Bone 81 (2015) 260–269

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Mechanical loading causes site-specific anabolic effects on bone following exposure to ionizing radiation☆

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ARTICLE INFO

Article history:
Received 12 March 2015
Revised 3 July 2015
Accepted 15 July 2015
Available online 18 July 2015

Keywords:
Mechanical loading
Ionizing radiation
Spaceflight
Histomorphometry
Cancellous bone microarchitecture
Cortical bone
Bone marrow
Osteoprogenitor
Stem cells

ABSTRACT

During spaceflight, astronauts will be exposed to a complex mixture of ionizing radiation that poses a risk to their health. Exposure of rodents to ionizing radiation on Earth causes bone loss and increases osteoclasts in cancellous tissue, but also may cause persistent damage to stem cells and osteoprogenitors. We hypothesized that ionizing radiation damages skeletal tissue despite a prolonged recovery period, and depletes the ability of cells in the osteoblast lineage to respond at a later time. The goal of the current study was to test if irradiation prevents bone accrual and bone formation induced by an anabolic mechanical stimulus. Tibial axial compression was used as an anabolic stimulus after irradiation with heavy ions. Mice (male, C57BL/6J, 16 weeks) were exposed to high atomic number, high energy (HZE) iron ions (^{56}Fe, 2 Gy, 600 MeV/ion) (IR, n = 5) or sham-irradiated (Sham, n = 5). *In vivo* axial loading was initiated 5 months post-irradiation; right tibiae in anesthetized mice were subjected to an established protocol known to stimulate bone formation (cyclic 9N compressive pulse, 60 cycles/day, 3 day/wk for 4 weeks). *In vivo* data showed no difference due to irradiation in the apparent stiffness of the lower limb at the initiation of the axial loading regimen. Axial loading increased cancellous bone volume by microcomputed tomography and bone formation rate by histomorphometry in both sham and irradiated animals, with a main effect of axial loading determined by two-factor ANOVA with repeated measure. There were no effects of radiation in cancellous bone microarchitecture and indices of bone formation. At the tibia diaphysis, results also revealed a main effect of axial loading on structure. Furthermore, irradiation prevented axial loading-induced stimulation of bone formation rate at the periosteal surface of cortical tissue. In summary, axial loading stimulated the net accrual of cancellous and cortical mass and increased cancellous bone formation rate despite prior exposure to ionizing radiation, in this case, HZE particles. Our findings suggest that mechanical stimuli may prove an effective treatment to improve skeletal structure following exposure to ionizing radiation.

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1. Introduction

Long-term habitation in the spaceflight environment presents significant biological challenges to organisms and negatively impacts skeletal integrity. Two aspects of the spaceflight environment of particular concern are the biomechanical challenges from weightlessness and biological damage from exposure to space radiation [1–3]. Whereas microgravity-induced bone loss has been studied extensively [4–9], consequences of exposure to space radiation on bone structure or metabolism are not as well understood.

During extended missions beyond the Earth's magnetosphere, astronauts may be exposed to both occasional solar particle events (SPEs) and continuous galactic cosmic radiation (GCR), which are substantially different than the dose, dose rate, and components typically encountered on Earth [10]. GCR and SPE include predominantly protons of various energies but also high atomic number and high-energy (HZE) particles, which are particularly damaging and therefore of particular concern. SPEs can deliver a relatively high dose (1–2 Gy) over a few days [11]. For a large SPE lasting 8-to-24 h, if unshielded, the whole body cumulative doses could reach the 1- to 2-Gy for protons depending upon the tissue site [12].

In contrast to space radiation exposures, radiotherapy typically is delivered in multiple doses (fractionation) of 1–2 Gy per fraction over a period of minutes for total doses up to 10–20 Gy or more [13], and consists of low-Linear Energy Transfer (LET) radiations, such as X-rays and gamma rays. LET is a measure of the average local energy deposited per unit length of distance traveled by a charged particle, and thus LET is a property of radiation that can yield different effects even at the same

☆ Conflict of interest statement: Nothing to disclose.

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http://dx.doi.org/10.1016/j.bone.2015.07.019
8756-3282/Published by Elsevier Inc.

dose. Healthy tissues, within the exposure field are estimated to absorb up to half of this dose [14], although radiation in some cases can exert substantial detrimental effects outside the exposure field via systemic mechanisms in what is described as abscopal effects [15]. Radiotherapy can lead to osteopenia via demineralization of bone, thinning of bones, sclerosis, and loss of trabecular connections in patients one year post-therapy [16–19], and can increase fracture risk [20–24]. Therefore, it is important to explore long-term recovery following exposure to radiation.

At space-relevant total doses (1–2 Gy), ionizing radiation exposure rapidly induces cancellous bone loss in rodents and acutely increases the number of osteoclasts that line cancellous surfaces contributing to skeletal fragility [25–28], but also may cause persistent damage to stem and progenitor cells for osteoblasts [29,30]. Exposure to low LET (^{137}Cs gamma, 2 Gy) radiation increases osteoclast surface by 46%, increases apoptosis of marrow cells, causes oxidative damage to lipids within mineralized tissue in 4-mo old male C57BL/6 mice, and decreases tibial cancellous bone volume fraction by 16% [31]. Similarly, repeated exposure to low LET X-ray radiation (from *in vivo* microCT imaging, 4 scans over a 2 week period) in 10-week old female C57BL/6 mice decreases cancellous bone volume fraction and increases trabecular spacing significantly [32].

Short duration experiments demonstrate that HZE particles may impair or lead to depletion of stem cells and osteoprogenitor cells in the osteoblast lineage [29], although low energy species such as gamma, protons and X-rays, also may exert detrimental effects and reduce osteoblast numbers if exposed to sufficiently high doses (2–20 Gy) [27]. High LET radiation (^{56}Fe, 1 GeV/nm, ≤2 Gy) inhibits *ex vivo* osteoblastogenesis from bone marrow precursors by 25%, increases osteoclast activity, and causes a decrement in tibial bone volume fraction after only 3 days in 4-mo old male C57BL/6 mice [29].

Low LET radiation (such as gamma or X-rays) can lead to an early increase in mineral apposition rate [28], followed by a reduction in mineralizing surface to bone surface in the cancellous compartment and endocortical surface *in situ*, reduced matrix formation [26,33], and later suppression of bone formation [34]. Total body gamma irradiation at high doses (5–6 Gy) significantly reduces the marrow cell density and stem cell population, which precedes deterioration of trabecular bone quality and quantity [35]. Irradiation of cultured osteoblasts and cell lines reduces collagen production, proliferation and differentiation and also increases sensitivity to apoptotic agents [36–39]. Furthermore, total body irradiation with high-LET ^{56}Fe or low-LET ^{137}Cs (gamma) reduces marrow cellularity and marrow-derived osteoprogenitors [29,31].

Taken together, the increased osteoclast activity and reduction of bone formation that is caused by irradiation may persistently suppress bone remodeling and turnover, resulting in osteopenia and deterioration of microarchitecture, which compromises material properties of the skeleton. In general, less is known about long-term effects of space-relevant doses of high LET radiation on the adult skeleton compared to short term effects, although our preliminary experiments indicate an inhibition of osteoblastogenesis 6 months following exposure to 2 Gy ^{56}Fe (data not shown), and substantive adverse effects of high doses in young animals [40].

We hypothesized that exposure to heavy ion radiation (2 Gy ^{56}Fe) persistently impairs bone formation at all skeletal surfaces despite a prolonged recovery period, depletes the ability of osteoblasts to respond to anabolic stimuli, and suppresses adaptive remodeling. In order to provide a rigorous test of our hypothesis, we selected the most damaging species (^{56}Fe) at a dose (2 Gy) that we showed previously to cause acute cancellous bone loss, impaired osteoblastogenesis in short duration experiments [29], and heightened skeletal fragility [41]. The *in vivo* axial loading model was used as an anabolic mechanical stimulus 6 months after exposure to HZE. *In vivo* cyclic compression of the mouse tibia is a well-established model and is commonly used to induce anabolic responses to mechanical stimuli in both cortical [42–44] and cancellous tissue [45,46]. Data from the current study indicate that, contrary to our hypothesis, both cancellous and cortical bone compartments did in fact respond to an anabolic stimulus 6 months post-radiation exposure by increasing trabecular thickness and improving cortical bone geometry, although deficits in periosteal bone formation appeared to persist.

2. Methods

2.1. Animals

Post-pubescent (16 weeks at time of irradiation), male, C57BL/6J mice (Jackson Labs) were housed individually and provided food (LabDiet 5001, St. Louis, MO) and water *ad libitum*, as described elsewhere [29]. Animal care and all experimental procedures were conducted in accordance with NASA Ames Research Center and Brookhaven National Laboratory IACUC rules and approvals.

2.2. Experiment design

Conscious mice (n = 12) were exposed to total body irradiation with high-LET iron ions (^{56}Fe, 2 Gy, 600 MeV/ion) at the NASA Space Radiation Laboratory, Brookhaven National Laboratory. Another group of mice (n = 12) were sham-irradiated, where they were placed in holders for the same duration as the experimental mice and taken inside the beam room without being exposed to radiation. After 2 weeks, mice were shipped to NASA Ames Research Center for the axial loading component of the study. A subset of mice from both irradiated and sham groups expired during transcontinental shipment, which occurred during the summer. Surviving animals appeared healthy and displayed normal behavior and recovery of body mass post-transport as compared to previous experiments (unpublished observations). Axial loading of the right tibia began 5 months post irradiation (n = 5 mice per group) at 9 months of age. This time point was selected based on our previous findings (*unpublished*) where exposure to 2 Gy iron impaired osteoblastogenesis from bone marrow cells 6 months later. Animals were delivered subcutaneous injections of fluorochrome bone label (calcein, 30 mg/kg) after the 9th and 12th loading sessions (2 and 9 days before euthanasia). Animals were euthanized by cardiac puncture and cervical dislocation. At the time of euthanasia, left and right tibiae were harvested for histomorphometric analyses.

2.3. Load–strain calibration

Load–strain calibration was performed to determine peak strain achieved during *in vivo* mechanical loading. Age-matched (9 mo. old, n = 6), male C57BL/6 mice were used to produce an *ex vivo* load–strain calibration curve. Mechanical strains were measured on the anteromedial tibial plateau at midshaft. Immediately following euthanasia, the musculature surrounding the right tibia was carefully retracted exposing only the medial diaphysis of the tibia, and a 120 Ω single-element strain gage (EA-06015DJ-120, Vishay Measurement Group, VMG) was bonded to the medial surface at diaphysis aligned with the bone's long axis with cyanoacrylate (M-Bond 200, VMG). Each gage was conditioned with a 0.8 V bridge excitation voltage and amplified with a gain of 300× using a signal conditioner (Model 2210, VMG). The amplified analog signal was digitized using an AD-DA board and fed into an oscilloscope (Tektronix, TDS 220). With the strain gage voltage zeroed, each tibia–fibula complex was axially loaded to increasing peak compressive loads (−1 N to −14 N, in 1 N increments) using a Bose Electroforce System 3220 (Bose Corporation, ElectroForce System Systems Group, Minnesota, USA). The average peak-to-peak voltage was recorded and voltage data was converted into strain using a conversion factor (microstrain/V) determined by electronic shunt calibration of the measuring hardware and confirmed by calculated strains (beam theory) using an aluminum cantilever.

Based on the strain calibration, −9N load induced +1288 ± 496 με at the medial surface of the tibia diaphysis and was used as an