

SIMULATED SPACE RADIATION AND WEIGHTLESSNESS: VASCULAR-BONE COUPLING MECHANISMS TO PRESERVE SKELETAL HEALTH

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Weightlessness causes a cephalad fluid shift and reduction in mechanical stimulation, adversely affecting both cortical and trabecular bone tissue in astronauts. In rodent models of weightlessness, the onset of bone loss correlates with reduced skeletal perfusion, reduced and rarified vasculature and lessened vasodilation, which resembles blood-bone symbiotic events that can occur with fracture repair and aging. These are especially serious risks for long term, exploration class missions when astronauts will face the challenge of increased exposure to space radiation and abrupt transitions between different gravity environments upon arrival and return. Previously, we found using the mouse hindlimb unloading model and exposure to heavy ion radiation, both disuse and irradiation cause an acute bone loss that was associated with a reduced capacity to produce bone-forming osteoblasts from the bone marrow. Together, these findings led us to hypothesize that exposure to space radiation exacerbates weightlessness-induced bone loss and impairs recovery upon return, and that treatment with anti-oxidants may mitigate these effects. The specific aims of this recently awarded grant are to: AIM 1 Determine the functional and structural consequences of prolonged weightlessness and space radiation (simulated spaceflight) for bone and skeletal vasculature in the context of bone cell function and oxidative stress. AIM 2 Determine the extent to which an anti-oxidant protects against weightlessness and space radiation-induced bone loss and vascular dysfunction. AIM 3 Determine how space radiation influences later skeletal and vasculature recovery from prolonged weightlessness and the potential of anti-oxidants to preserve adaptive remodeling.

Our experimental approach will be to use post-pubertal (4-mo old), male C57Bl6/J mice exposed to hindlimb unloading by tail traction to simulate weightlessness, and also exposed via total body irradiation to protons and heavy ions at NSRL to simulate space radiation. To establish conditions and radiation dose-dependence for the proposed experiments, mice were irradiated with either protons (150MeV/ μ ; dose rate 20-50 cGy/min) or ⁵⁶Fe particles (600MeV/ μ ; dose rate 5-55 cGy/min) at 5, 10, 50 or 200 cGy (as a positive control), then tissues were harvested after 5 wk. Bone marrow (tibia and femur) was collected and plated in osteoblastogenic media consisting of α -MEM supplemented with 15% fetal calf serum, 50 μ g/mL ascorbate and 10mM β -glycerophosphate until mineralization of nodules was detected (~20 days, n=5-6/group). Nodule formation was assessed as the percentage of mineralized plating surface in digital scans of the wells. Proximal tibiae were scanned by 3-D microcomputed tomography (SkyScan 1174, 7 μ m/pixel) to evaluate trabecular microarchitecture. A threshold of p<0.05 was set for significance (ANOVA, Tukey-Kramer post-hoc). All groups had comparable body weights. ⁵⁶Fe exposure at low doses of radiation (10cGy) reduced nodule formation by 68% relative to controls. There was a large variability in osteoblastogenesis from proton-irradiated mice and statistically significant differences were not observed. Microarchitectural analyses revealed that 50cGy reduced fractional bone volume to total volume (BV/TV) by 16% and 200cGy reduced BV/TV by 31%. Doses lower than 50cGy had no significant effect. Proton irradiation had similar, though lesser effects compared to ⁵⁶Fe. Protons at 50cGy reduced BV/TV by 11% and 200cGy by 22%. Comparison with values obtained from mice at the time of irradiation revealed that the changes in BV/TV were due to a net decline in bone mass after irradiation, i.e. loss of bone tissue. Consistent with these findings, microarchitectural changes included a reduction in trabecular number and an increase in the ratio of rods-to-plates (Structural Model Index) but no change in trabecular thickness relative to age-matched controls. Thus, based on an 18% decrement in BV/TV, the Relative Biological Effectiveness (RBE) of ⁵⁶Fe to proton at these energies was estimated to be 1.8-2.0.

In conclusion, we have established conditions for evaluating bone loss due to simulated spaceflight (weightlessness, radiation). Future experiments will entail exposure to both protons and ⁵⁶Fe in combination with hindlimb unloading to determine basic mechanisms, risks and possible countermeasures for bone loss in astronauts.

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