

EFFECTS OF HEAVY-ION IRRADIATION ON EX VIVO OSTEOBLASTOGENESIS, OXIDATIVE STRESS RESPONSE, AND CANCELLOUS BONE MICROARCHITECTUREJ.S. Alwood¹, L. H. Tran¹, A.Kumar¹, C.L. Limoli², and R.K. Globus¹¹NASA Ames Research Center, Moffett Field, CA, ²University of California, Irvine, CA

Prolonged spaceflight causes degeneration of skeletal tissue with incomplete recovery even after return to Earth. We hypothesize that exposure to heavy-ion radiation, a component of Galactic Cosmic Radiation, damages osteoblast progenitors via oxidative stress and that damage may contribute to imbalanced bone remodeling during long-duration space travel beyond the protection of the Earth's magnetosphere.

Male, 16 week-old C57Bl/6J mice were exposed to high-LET (⁵⁶Fe, 600MeV) radiation using either low (5 or 10cGy) or high (50 or 200cGy) doses at the NASA Space Radiation Lab and were euthanized 1, 3-4, 7, or 35 days later. Bone structure was quantified by microcomputed tomography (6.8 μm pixel size), the redox microenvironment of the marrow was characterized by measuring the total antioxidant capacity of the extracellular fluid (bone marrow plasma) biochemically (Kit #TA02, Oxford Biomedical), and the intracellular concentration of reactive oxidative or nitrosative species was assessed in freshly isolated marrow cells using membrane-permeable, fluorogenic dyes. To assess osteoblastogenesis, adherent marrow cells were cultured *ex vivo*, then growth and mineralized nodule formation quantified by imaging, and gene expression analyzed by RT-PCR.

Interestingly, 3-4 days post-exposure, fluorogenic dyes reflecting cytoplasmic generation of reactive nitrogen species (DAF-FM diacetate) or reactive oxygen species (CM-H2DCFDA) revealed that irradiation (50cGy) reduced free radical generation (20-45%) in marrow cells compared to sham-irradiated controls. In contrast, use of a dye that shows relative specificity for mitochondrial superoxide generation (MitoSOX) revealed that irradiation caused an 88% increase compared to controls. A high dose of radiation (200cGy) transiently decreased the antioxidant capacity of the marrow plasma at 1 and 3 days post-irradiation, indicative of depleted antioxidant defenses in the marrow microenvironment. One week after radiation exposure (50cGy), reactive oxygen and nitrogen species generation by marrow cells remained lower (24%) than sham-irradiated controls. After one month, high dose irradiation (200cGy) caused an 86% decrement in *ex vivo* nodule formation and growth and a 16-31% decrement in bone volume to total volume ratio and trabecular number (50, 200cGy) compared to controls. High dose irradiation also (200cGy) up-regulated expression of a late osteoblast marker (BGLAP) and select genes related to oxidative metabolism (Catalase) and DNA damage repair (Gadd45). In contrast, lower doses (5, 10cGy) did not affect bone structure or *ex vivo* osteoblastogenesis, but did down-regulate iNOS by 0.56 fold.

Thus, exposure to both low and high doses of heavy-ion radiation caused time-dependent changes in the oxidative stress response within marrow cells, but only high doses (50, 200cGy) inhibited osteoblastogenesis and caused cancellous bone loss. We conclude that total body irradiation with a high dose of heavy ions leads to oxidative stress within the bone marrow despite compensatory changes in cellular redox state; this may contribute to damaging stem and progenitor cells of the osteoblast lineage and consequently, produce long term deficits in skeletal remodeling.

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