LATE EFFECTS OF HEAVY-ION IRRADIATION ON EX VIVO OSTEOBLASTOGENESIS AND CANCELLOUS BONE MICROARCHITECTURE.

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Prolonged spaceflight causes degeneration of skeletal tissue with incomplete recovery even after return to Earth. We hypothesize that heavy-ion irradiation, a component of Galactic Cosmic Radiation, damages osteoblast progenitors and may contribute to bone loss during long duration space travel beyond the protection of the Earth's magnetosphere. Male, 16 week-old C57BL6/J mice were exposed to high-LET (56-Fe, 600MeV) radiation using either low (5 or 10cGy) or high (50 or 200cGy) doses at the NASA Space Radiation Lab and were euthanized 3-4, 7, or 35 days later. Bone structure was quantified by microcomputed tomography (6.8 μm pixel size) and marrow cell redox assessed using membrane-permeable, free radical-sensitive fluorogenic dyes. To assess osteoblastogenesis, adherent marrow cells were cultured ex vivo, then mineralized nodule formation quantified by imaging and gene expression analyzed by RT-PCR. Interestingly, 3-4 days post-exposure, fluorogenic dyes that reflect cytoplasmic generation of reactive nitrogen/oxygen species (DAF-FM Diacetate or CM-H2DCFDA) revealed irradiation (50cGy) reduced free radical generation (20-45%) compared to sham-irradiated controls. Alternatively, use of a dye showing relative specificity for mitochondrial superoxide generation (MitoSOX) revealed an 88% increase compared to controls. One week after exposure, reactive oxygen/nitrogen levels remained lower (24%) relative to sham-irradiated controls. After one month, high dose irradiation (200 cGy) caused an 86% decrement in ex vivo nodule formation and a 16-31% decrement in bone volume to total volume and trabecular number (50, 200cGy) compared to controls. High dose irradiation (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 nodule formation, but did down-regulate iNOS by 0.54-0.58 fold. Thus, both low- and high-doses of heavy-ion irradiation cause time-dependent, adaptive changes in redox state within marrow cells but only high doses (50, 200cGy) inhibit osteoblastogenesis and cause cancellous bone loss. We conclude space radiation has the potential to cause persistent damage to bone marrow-derived stem and progenitor cells for osteoblasts despite adaptive changes in cellular redox state.