System Re-set: High LET Radiation or Transient Musculoskeletal Disuse Cause Lasting Changes in Oxidative Defense Pathways within Bone

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BACKGROUND
Space exploration exposes the musculoskeletal system to unique challenges. Evidence from both the clinic and animal studies shows that therapeutic doses of radiation or reduced weight bearing cause bone loss and impair mechanical properties, leading to elevated fracture risk, i.e. osteoporosis. High doses (≥1 Gy) of both high and low LET total body irradiation (IR) cause bone loss within high turnover, cancellous tissue of adult mice, in a rapid and probably irreversible manner. We previously reported that in the short term, treatment with an anti-oxidant prevents short-term cancellous bone loss caused by IR, and in the long term, space-relevant irradiation impairs skeletal remodeling and recovery from disuse.

HYPOTHESIS
Irradiation alone, or combined with disuse, leads to persistent changes in skeletal expression of stress-related genes relevant to p53 signaling and oxidative metabolism, reflecting a re-setting of key molecular signaling pathways.

METHODS
The rodent hindlimb unloading (HU) model was used to simulate weightlessness, and IR with heavy ions (56Fe, 1 GeV) at NSRL/BNL was used to simulate exposure to Galactic Cosmic Radiation. Male, 4-mo old, C57Bl6/J mice were normally loaded (NL) or HU (2 wk), then each group was irradiated (sham, 50cGy during unloading). HU mice were released to ambulate normally 3 d after irradiation, all mice were transferred to Ames Research Center and tissues harvested 6.5 months later. RNA was purified from intact tibiae (includes both bone and marrow) using RNeasy RNA purification kit (Qiagen), then cDNA synthesized using RT2 First Strand kit (SABiosciences) (n=4/group). Quantitative real time PCR was performed using real time profiler PCR array to evaluate expression of 84 mouse genes relevant to p53 or oxidative defense pathways (SABiosciences). Relative expression was calculated using the comparative threshold cycle method (DeltaDeltaCt) and data from each group compared to NL/0Gy controls using Student’s t-test (SABiosciences Data Analysis software), with P<0.05 accepted as significant. Serum was analyzed for nitrite/nitrate by the Greiss reaction as an indicator of nitrosyl stress.

RESULTS AND DISCUSSION
Six months post-IR, there were no notable changes in skeletal expression of 84 principal genes in the p53 signaling pathway due to low dose IR (0.5Gy), HU, or both. In contrast, numerous genes relevant to oxidative stress were regulated by the treatments, typically in a direction indicative of increased oxidative stress and impaired defense. IR and HU independently reduced (between 0.46 to 0.88 fold) expression levels of Noxa1, Gpx3, Prdx2, Prdx3, and Zmynd17. Surprisingly, transient HU alone (sham-irradiated) decreased expression of several redox-related genes (Gpx1,Gstl1, Prdx1, Txnrd2), which were not affected significantly by IR alone. Irradiation increased (1.13 fold) expression of a gene responsible for production of superoxides by neutrophils (NCF2). Of interest, only combined treatment with HU and IR led to increased expression levels of Ercc2, (1.19 fold), a DNA excision repair enzyme. Differences in gene expression levels may reflect a change in gene expression on a per cell basis, a shift in the repertoire of specific cell types within the tissue, or both. Serum nitrite/nitrate levels were elevated to comparable levels (1.6-fold) due to IR, HU or both, indicative of elevated systemic nitrosyl stress.

CONCLUSIONS
The magnitude of changes in skeletal expression of oxidative stress-related genes six months after irradiation and/or transient unloading tended to be relatively modest (0.46-1.15 fold), whereas the p53 pathway was not affected. The finding that many different oxidative stress-related genes differed from controls at this late time point implicates a generalized impairment of oxidative defense within skeletal tissue, which coincides with both profound radiation damage to osteoprogenitors/stem cells in bone marrow and impaired remodeling of mineralized tissue.

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