OXIDATIVE STRESS AND SKELETAL HEALTH WITH LOW DOSE, IONIZING RADIATION

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Osteoporosis profoundly affects the aging U.S. population and exposure to high doses of radiation causes bone loss similar to age-related osteoporosis, although the influence of low dose radiation exposures is not known. The central hypothesis of our DOE project (NASA supplement) is that low doses of radiation modulate subsequent skeletal degeneration via oxidative pathways. Our working hypothesis is that a prior exposure to low dose radiation regulates oxidative metabolism within bone and contributes to bone loss caused either by subsequent high, challenge doses of radiation or by aging.

HZE source: Because astronauts are exposed to radiation from GCR and solar particle events in microgravity, we conducted experiments to determine if unloading during irradiation with a relatively low dose of high LET radiation damages skeletal progenitor populations and affects skeletal aging. Four-month old, male, C57Bl6/J mice were irradiated with ^{56}Fe (1GeV/amu) at doses of 0 (sham), 50 or 200 cGy (at NSRL/BNL) then tissues harvested 6.5 months later. Additional groups of mice at the time of irradiation were subjected to hindlimb unloading (HU), which simulates weightlessness and bedrest; these mice were then released to ambulate normally three days after exposure. Irradiation with 50 cGy of normally loaded (NL) mice did not affect the fractional bone volume/total volume (BV/TV), an index of bone density measured by 3D microcomputed tomography, in the high turnover, cancellous tissue 6.5 months later. In contrast, irradiation with the same dose (50 cGy) of mice that were transiently hindlimb unloaded at the time, caused a 20% reduction in BV/TV relative to NL mice ($P<0.001$ by 1-factor ANOVA, Tukey-Kramer post hoc test). The bone loss reflected both complete loss of trabeculae and thinning of existing trabeculae. Irradiation with 50 or 200 cGy also dramatically reduced the generation of new osteoblasts from bone marrow ex vivo (osteoblastogenesis). As marrow cell cultures from irradiated and control mice did not grow at the same rate, the observed defect in osteoblastogenesis appears due to impaired cell division.

Photon source: Previously we demonstrated high doses of total body irradiation (^{137}Cs, 1 and 2 Gy) increase the numbers of bone-resorbing osteoclasts leading to tissue loss in adult mice. Because there is a net increase in osteoclast numbers on cancellous bone surfaces within a short period of time (3 days), the rapid bone loss observed can be explained by a stimulation of osteoclastogenesis from monocyte/macrophage precursors within the haematopoietic lineage. The monocyte/macrophage cell line (RAW264.7) or primary bone-marrow-derived cells can be used to study the generation of new osteoclasts. Multinucleated osteoclast-like cells that display enzymatic activity for tartrate-resistant acid phosphatase (TRAP) are quantified directly using image analysis techniques. High (challenge) doses of ^{137}Cs cause a 1.8-3 fold increase in osteoclastogenesis from the RAW264.7 cell line. This radiation-induced increase in osteoclast numbers was only observed when the culture medium was supplemented with low concentrations of the obligate osteoclast differentiation factor, Receptor Activator for

NFκB Ligand (RANKL). When the cell line or primary bone marrow cells were irradiated in the absence of RANKL, the expected dose-dependent decline in cell number was observed, reflecting increased cell death and/or reduced proliferation. Thus, RANKL appeared to switch the radiation responses of macrophage/monocyte cell precursors from damaging effects on cell growth to stimulatory effects on osteoclast differentiation. These experiments established the high doses that damage osteoclast precursors in preparation for adaptive radiation experiments. To determine whether or not low doses of radiation cause adaptive responses that influence cell behavior triggered by high doses ('challenge'), RAW264.7 cells were irradiated with 2 cGy prior to challenge 24 hrs later with sham or 2Gy irradiation. The adaptive dose had little effect on osteoclastogenesis, although there was a modest, non-significant increase relative to cultures treated with the challenge dose alone (1.3 fold). Additional experiments exploring the possible adaptive influence of low dose radiation *in vivo* are in progress.

In sum, we showed that skeletal aging, as assessed by trabecular microarchitecture, is adversely affected by prior exposure to radiation; progenitors and precursors from both osteoblasts and osteoclasts lineages appear to contribute to observed bone loss. Both the *in vitro and in vivo* approaches developed will be useful for future studies to define the mechanisms and the long-term skeletal effects of exposure to low doses of radiation.

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