Consequences of Low-Dose HZE Irradiation in the Cortical Bone of Aged Mice

J. S. Alwood[1], L. H. Tran[1], A. Kumar[1], D. Hilton[1], S.Y. Choi[2], S. Torres[1], C. L. Limoli[3], R. K. Globus[1]
[1]Bone and Signaling Laboratory, NASA Ames Research Center, Mail-Stop 236-7, Moffett Field, CA, 94035, USA, [2]Lockheed Martin, Mail-Stop 236-7, Moffett Field, CA, 94035, USA, and [3]Department of Radiation Oncology, University of California Irvine, Irvine, CA, 92697, USA.

During spaceflight, astronauts are exposed to HZE radiation at relatively low doses, which may result in acute and late changes in skeletal structure, strength, and the ability of bone cells to perform routine structural maintenance. In the acute response of rodents, space radiation causes osteopenia through increased bone resorption, with changes occurring mostly within the metabolically active cancellous tissue. During normal aging of humans, cancellous alterations, including strut thinning and increased porosity, may precede or proceed more rapidly, than cortical changes. Whether space radiation causes later changes to cortical bone, which is critical to bone's mechanical integrity, is not well understood. Therefore, we hypothesized that HZE radiation alters cortical tissue and produces a reduced state of cortical bone remodeling. To test this, we collaborated with UTMB (R. Ullrich) to study the late effects of 56Fe particulate irradiation (600 MeV, 100 cGy/min) as a model of space radiation. Conscious C3H mice (male, 10 wks, n = 100-200/group) receive total body irradiation at NSRL/BNL with 10, 20, 40, and 100 cGy. Prior to tissue harvest, cohorts of mice were examined to exclude those with palpable tumors or visible lesions. Two years after irradiation, mice selected from these cohorts (n = 8-17/dose) were euthanized and femora collected for analysis of skeletal structure with microcomputed tomography (7μm pixel size), an indicator of bone strength, and bone marrow cells were cultured ex vivo in media containing factors promoting osteoclastogenic or osteoblastogenic differentiation. For osteoclastogenesis, marrow was cultured on a thin layer of calcified substrate to quantify bone-resorption capacity of osteoclast-like cells. For osteoblastogenesis, marrow was cultured up to 30 days until mineralized nodules were formed. The alizarin-positive nodule area was quantified by image analysis and normalized to the well area. During dissection, approximately 50% of the control mice had visible lesions. The cohort with the highest dose, 100 cGy, had too many lesions and tumors to include in subsequent analyses.

In the aged control mice, femoral diaphyses displayed cortical porosity (13.5 % ± 1.3 % of the total volume) adjacent to the endosteal surface. Irradiation at 20 and 40 cGy, but not 10 cGy, increased cortical bone volume of the femoral diaphysis by 10% compared to controls, mainly due to a reduced pore and void volume of the marrow cavity. However, irradiation did not alter the periosteal or endosteal perimeters, nor did it affect femoral length, relative to age-matched controls, indicating radial and longitudinal growth was not impeded by irradiation. After 7 days of osteoclastogenic marrow culture, control mice showed low amounts (< 5% of the substrate area) of in vitro resorption (osteoclasts defined by being TRAP-stain positive and having > 2 nuclei), with no changes due to 10 cGy irradiation. However, the amount of resorbed area correlated ($r = 0.50$, $p = 0.07$) with cortical porosity, indicating a relationship between the resorption capacity of marrow osteoclasts and the topography of the endosteal surface. After 30 days of osteoblastogenic marrow culture, control mice showed low amounts of nodule formation (< 5% of well area), with no changes due to any dose of radiation, suggesting a relatively low population of osteoprogenitors due to age alone. Taken together, these data support HZE radiation causing reduced remodeling within cortical bone. We speculate that increased bone volume in the cortex following irradiation results from reduced osteoclast activity due to persistent radiation-induced damage to stem or myeloid-lineage cells that give rise to osteoclasts. In conclusion, the late effects of space irradiation on cortical bone structure, though mild, may impair normal tissue remodeling and ultimately alter bone strength.