ABSTRACT

Future long-duration space exposure beyond low Earth orbit will increase human exposure to space radiation and microgravity conditions as well as associated risks to skeletal health. In animal studies, radiation exposure (>1 Gy) is associated with pathological changes in bone structure, enhanced bone resorption, reduced bone formation and decreased bone mineral density, which can lead to skeletal fragility. Definitive measurements and detection of bone loss typically require large and specialized equipment which can make their application to long duration space missions logistically challenging. Towards the goal of developing non-invasive and less complicated monitoring methods to predict astronauts’ health during spaceflight, we examined whether radiation-induced gene expression changes in skin may be predictive of the responses of skeletal tissue to radiation exposure. We examined oxidative stress and growth arrest pathways in mouse skin and long bones by measuring gene expression levels via quantitative polymerase chain reaction (qPCR) after exposure to total body radiation (IR). To investigate the effects of radiation on gene expression, we used skin and femora (cortical shaft) from the following treatment groups: control (normally loaded, sham-irradiated), and IR (0.5 Gy 17MeV/n and 0.5 Gy 42MeV/n, respectively). Gene expression was measured using microcomputed tomography (microCT) and immunohistochemistry (IHC).

RESULTS

In skin, FGF18 transiently increased 24 hours after irradiation (IR). There was no significant difference in expression levels between IR and Control groups at 24 hours post IR. No effects on gene expression were detected 1 day or 11 days post-IR.

CONCLUSION

FGF18 expression in skin and MCP-1 expression in bone were strongly correlated 1 day after exposure to radiation (P<0.002, r=0.8779). Further, microcomputed tomography analysis of tibiae from animals at a later time (11 days) showed reduced cancellous bone volume/total volume (-21.7%) at 11 days post-IR. These results suggest that measurements of early radiation-induced changes in FGF18 gene expression in skin may have value for predicting subsequent loss of cancellous bone mass. Further research may lead to the development of a relatively simple diagnostic tool for bone loss, with the advantage that hair follicles and skin are relatively easy to acquire from human subjects.

METHODS

Animal: Male C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME), 16 weeks of age

Experimental group:
- Control (Cont)
- Radiation exposure (Rad) – 1 day and 11 days after total body irradiation

Mice were exposed to a single dose of radiation consisting of 1 Gy of total body irradiation (0.5 Gy 17MeV/n and 0.5 Gy 42MeV/n) at a dose rate of 5 Gy/min (HFe) , 3 Gy/min (H) at the NASA Space Radiation Laboratory beamline at Brookhaven National Laboratory (BNL).

Extraction of RNA:
Total RNA was extracted from skin and femur (flushed of marrow) using Trizol.

Gene expression analysis:
Quantitative polymerase chain reaction (qPCR) was performed for the following genes: Cdkn1a, FoxO3, SOD1, Gadd45g, Trp53, FGF18, Nfe2l2 (for skin) and MCP-1, Nfe2l2, Rankl (for femur).

VALUES ARE NORMALIZED TO EXPRESSION LEVELS OF L19. (n=5/group)

Microcomputed tomography (MicroCT):
Tibiae were scanned by microCT. Bone volume per total volume (BV/TV) was calculated. (n=6/group)

Immunohistochemistry in skin:
The expression of FGF18 was analyzed using monoclonal antibodies specific for FGF18.

The avidin-biotin immunohistochemical procedure was used for the localization of primary antibody binding according to manufacturer’s instructions (ABC kit, Santa Cruz Biotechnology). (n=5/group)

Statistics:
Data shown are means ±S.D. Student T-test was performed, and P<0.05 accepted as significant.