Effects of hindlimb unloading and ionizing radiation on murine gene expression in skin and bone

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ABSTRACT

Long duration spaceflight causes a negative calcium balance and reduces bone density in astronauts. The underlying mechanisms of spaceflight-induced bone loss and the possible influences of both microgravity and radiation are not fully understood although emerging evidence suggests that these two factors may interact to result in increased bone loss. Previously, gene expression analysis of hair follicles from astronauts, as well as skin from space-flown mice, revealed changes in the expression of genes related to DNA damage and oxidative stress responses. These results resemble the responses of bone to spaceflight-like radiation and simulated weightlessness by hindlimb unloading (HU). Hence in this study, we initiated studies to determine whether skin can be used to predict the responses of bone to simulated microgravity and radiation. 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). To investigate the effects of irradiation and/or HU on gene expression, we used skin and femora (cortical shaft) from the following treatment groups: control (normally loaded, sham-irradiated) (CT), hindlimb unloading (HU), ^{56}Fe radiation (IR) and both HU+IR. Animals were euthanized 11 days post-IR, and results were analyzed by 2-way ANOVA. In skin samples, Cdkn1a was decreased to the same extent in HU and HU+IR (47% of CT). In addition, HU reduced FoxO3 expression (46% of CT) and IR increased Gadd45g expression 135% compared CT in skin. In contrast, HU increased skeletal FoxO3 expression 31% compared to CT levels. These results suggest that radiation and simulated weightlessness regulated similar oxidative stress and cell cycle arrest genes in both skin and bone, although the time course and direction of changes may differ. This 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.

INTRODUCTION

Background:

- Simulated space radiation induces bone loss.
- Skin is one of the most radiation-responsive organs.

Previous research at JAXA (Japanese Space Agency)

We performed gene expression analysis on hair follicles from astronauts as well as skin from space-flown mice. We detected changes in the expression of genes related to DNA damage and oxidative responses. These results appear to mimic the known responses of bone to spaceflight-like radiation and simulated microgravity.

Advantages of using samples:

Hair follicles and skin are relatively easy to acquire from subjects. Bone on the other hand, requires special imaging devices to be analyzed for structure and sampling for biochemical analyses is invasive.

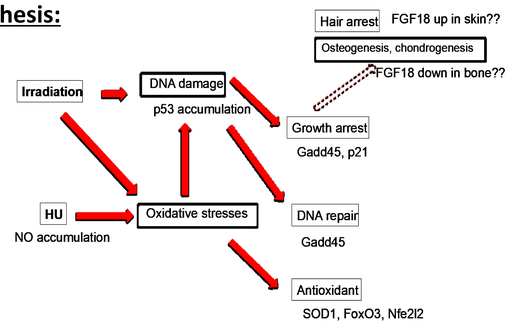
Purpose of this study:

Determine if skin can be used to predict the responses of bone to simulated microgravity and radiation

Long-term goal:

Develop a relatively simple diagnostic tool for bone loss by analyzing skin

Hypothesis:



- FoxO3 inhibits apoptosis and protects from oxidative stress.
- Cdkn1a arrests cell cycle and inhibits DNA replication.
- Gadd45 is important in cell cycle regulation and DNA repair

METHODS

Animal:

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

Experimental group:

- Control (Con)
- Radiation exposure (Rad)[1)]
- Hindlimb unloaded (HU)[2)]
- Combined HU and Rad exposure (HU-Rad)

Extraction of RNA:

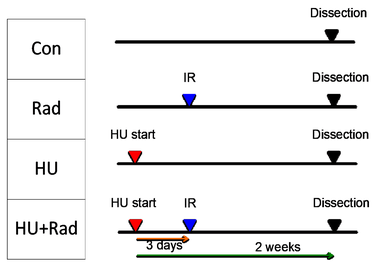
Total RNA was extracted from skin and femur (flushed of marrow) using Trizol.

Gene expression analysis:

Quantitative polymerase chain reaction (qPCR) was performed for the following genes: Cdkn1a, FoxO3, SOD1, Gadd45g, Trp53, FGF18, Nfe2l2. Values are normalized to expression levels of L19.

Statistics:

Two-way analysis of variance was performed with Rad and HU as main effects. Post hoc test for significant interaction effects was performed by Tukey-Kramer test.

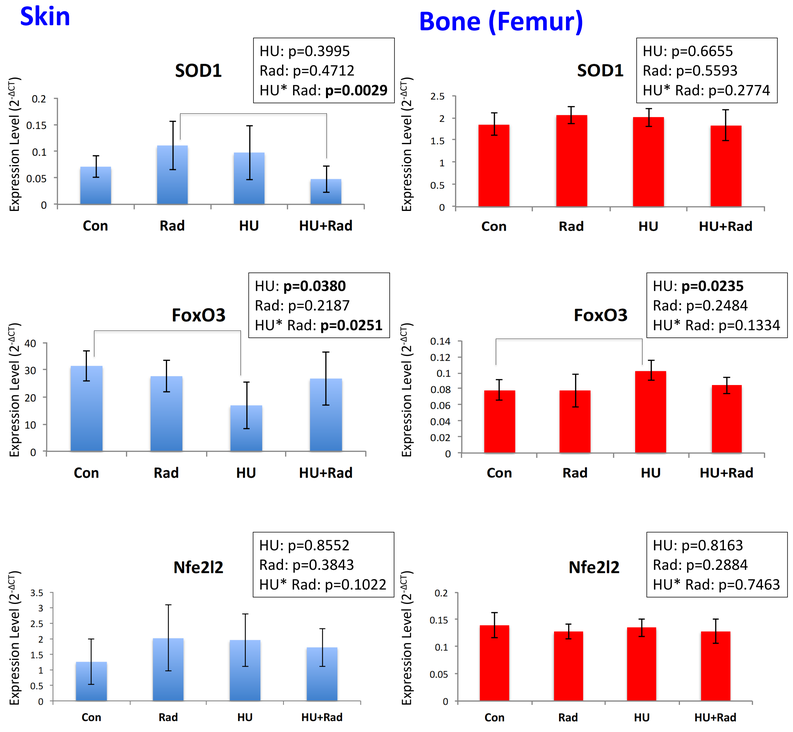


[1)] Mice were exposed to a single dose of radiation consisting of 1 Gy of ^{56}Fe ions (600 MeV/nucleon, LET 150 keV/µm) at a dose rate of 10cGy/min at the NASA Space Radiation Laboratory beamline at Brookhaven National Laboratory (BNL). Irradiation of HU-Rad mice took place 3 days after the initiation of hindlimb unloading.

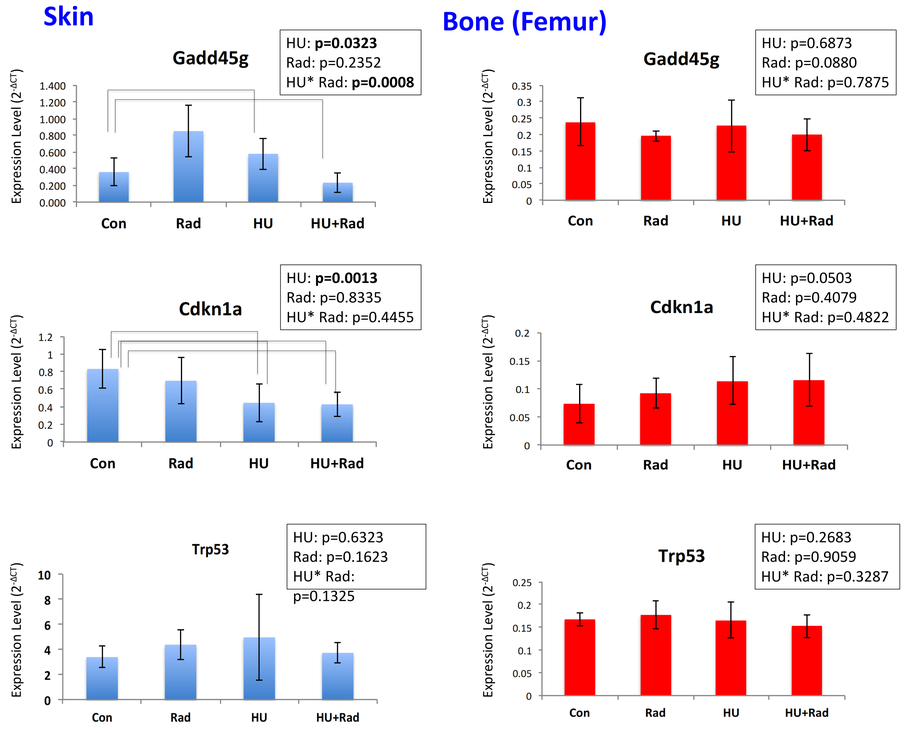
[2)] Mice were hindlimb unloaded via tail traction for 13-16 days until the time of euthanasia.

RESULTS

Responses related to oxidative stress

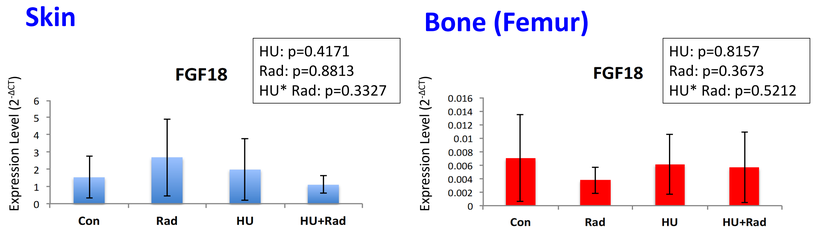


Responses related to DNA repair



◆**HU treatment decreased FoxO3 expression in skin, but increased expression in bone.** Expression of this gene is important for defense against oxidative stress caused by various stressors, including aging.

Responses related to hair growth or osteogenesis



In skin:

◆**HU treatment decreased Cdkn1a expression, and increased Gadd45g expression compared to Controls.** This suggests that HU treatment may affect cell cycle and apoptosis in skin.

◆**Exposure to radiation affected expression of FoxO3, SOD1 and Gadd45g differently in HU animals (HU+ Rad) compared to animals that ambulated normally (Rad)** (see interaction term)**.** Thus, we conclude simulated weightlessness appeared to blunt the effects of ionizing radiation on expression of oxidative stress and cell cycle arrest genes.

◆**Expression levels of FGF18, Nfe2l2 and Trp53 were not substantially changed by any treatment.**

L19 was used as internal control. Data are presented as Mean ± SD.

CONCLUDING COMMENTS

In both skin and bone, simulated weightlessness and ionizing radiation regulated FoxO3 expression, although opposite in direction. We report here for the first time that simulated weightlessness alone regulated gene expression of skin (FoxO3, Cdkn1a and Gadd45g). In this study, we analyzed only a single time point; possible common effects in skin and bone may occur at different time points after treatment. 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.

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