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Assessment of Dose-Dependent Endocrine and Immune Responses to Simulated Ionizing Radiation

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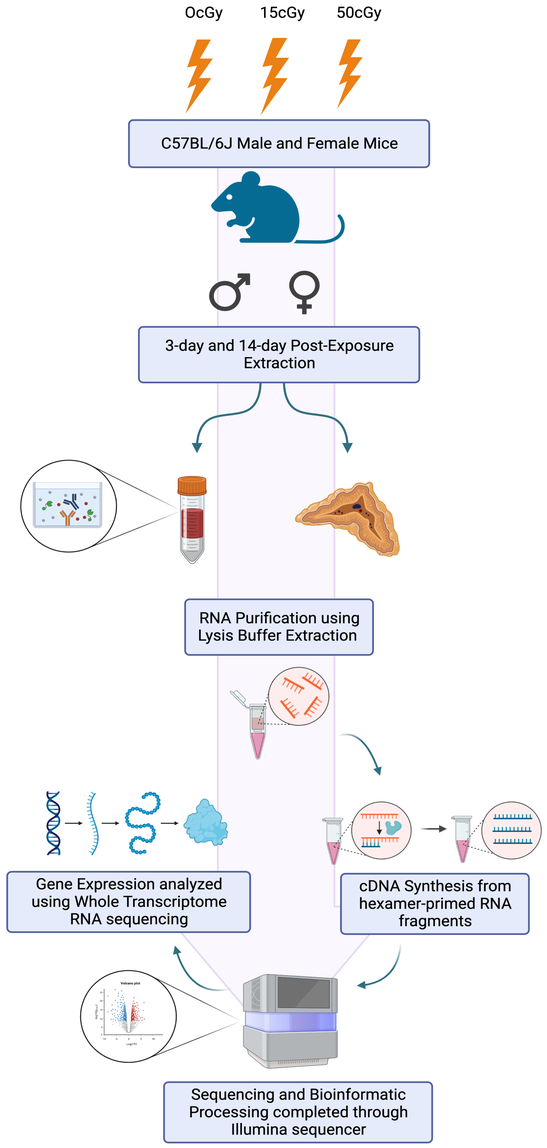
Overview

The Hypothalamic-pituitary-adrenal axis can regulate immune responses to counteract stressful stimuli in maintaining homeostasis within the body. Cosmic ionizing radiation is an innate risk within the space environment, and it is known to cause direct DNA damage and indirectly impact cellular function, transduction, and communication process. Assessment of different physiological systems and their interactions are important to consider for mitigation strategies in spaceflight. The degree of ionizing radiation and relative biological effectiveness is an open question as it pertains to immune and endocrine responses. Therefore, this study will assess the dose-dependent responses of immunity and adrenal function to cosmic ionizing radiation. For this, male and female *C57BL/6J* mice were exposed to simulated, simplified five-ion galactic cosmic ray (GCR) radiation at 5cGy, 15cGy, and 50cGy. Blood and tissues were collected two-weeks post exposure and inflammatory biomarkers and hormone biochemical pathways were characterized by whole transcriptome RNA sequencing. Results displayed differential transcriptomic profiles for each condition and sex, indicating complex responses and networks are generated from different doses of ionizing radiation. Careful consideration of unique profiles highlights the current need for personalized medicine requirements for astronauts exposed to similar doses on exploration missions.

Methodology



- Organ weights collected at 14-days post irradiation.
- ELISA performed at 3-days post irradiation to determine endocrine hormone production.
- Whole transcriptome RNA sequencing of adrenals used to measure differential gene expression between 50cGy and sham controls in each sex.

Data

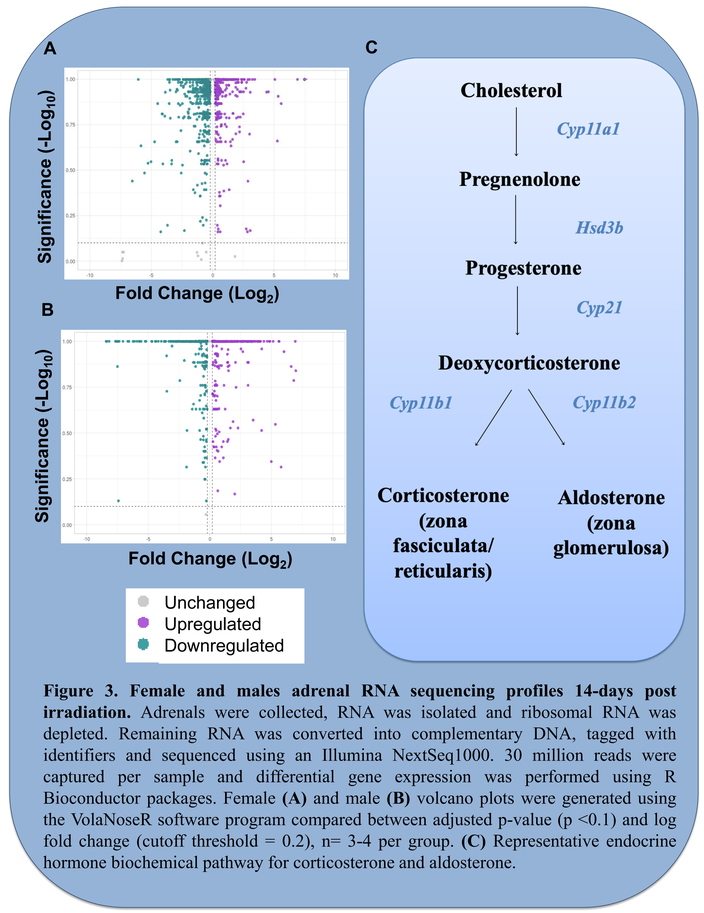


Figure 3. Female and males adrenal RNA sequencing profiles 14-days post irradiation. Adrenals were collected, RNA was isolated and ribosomal RNA was depleted. Remaining RNA was converted into complementary DNA, tagged with identifiers and sequenced using an Illumina NextSeq1000. 30 million reads were captured per sample and differential gene expression was performed using R Bioconductor packages. Female **(A)** and male **(B)** volcano plots were generated using the VolaNoseR software program compared between adjusted p-value ($p < 0.1$) and log fold change (cutoff threshold = 0.2), n= 3-4 per group. **(C)** Representative endocrine hormone biochemical pathway for corticosterone and aldosterone.

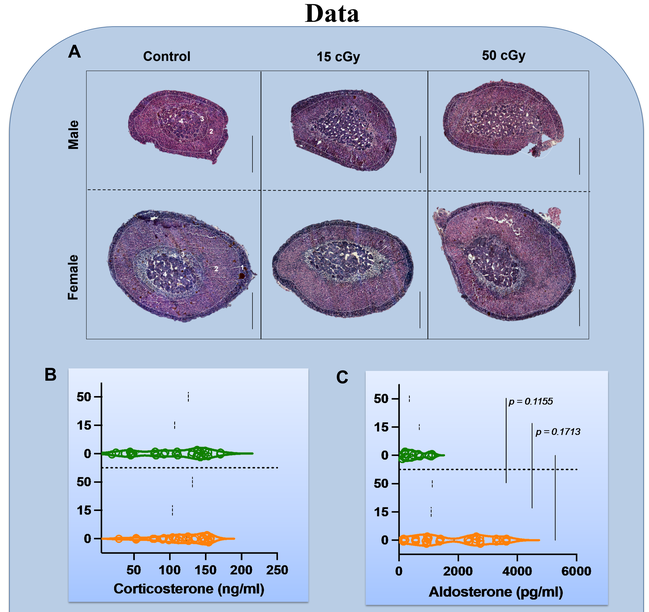


Figure 1. Sex- and dose-specific adrenal gland architecture and hormones production profiles post-irradiation. Male and female mice were exposed to GCR_{sim} (0, 15, and 50 cGy) and at 14-days post-IR adrenal glands were isolated, fixed, paraffin-embedded, sectioned (10 µm) and stained with hematoxylin and eosin for cellular architecture identification **(A)**. Three-days post-IR blood was isolated and enzyme-linked immunosorbent assays (ELISA) were performed for adrenal hormone characterization, including corticosterone **(B)** and aldosterone **(C)**. Scale bars = 450 µm. Sectioned regions highlight: 1. Zona glomerulosa; 2. Zona fasciculata; 3. Zona reticularis; 4. Medulla. Females (orange) and males (green) are displayed. Parametric or nonparametric analyses were performed with post hoc analyses as describe in the methods section. Data represent ±SEM, $p^* < 0.05$, n= 10-12 per group. Technical replicates (n=3) were performed with each ELISA.

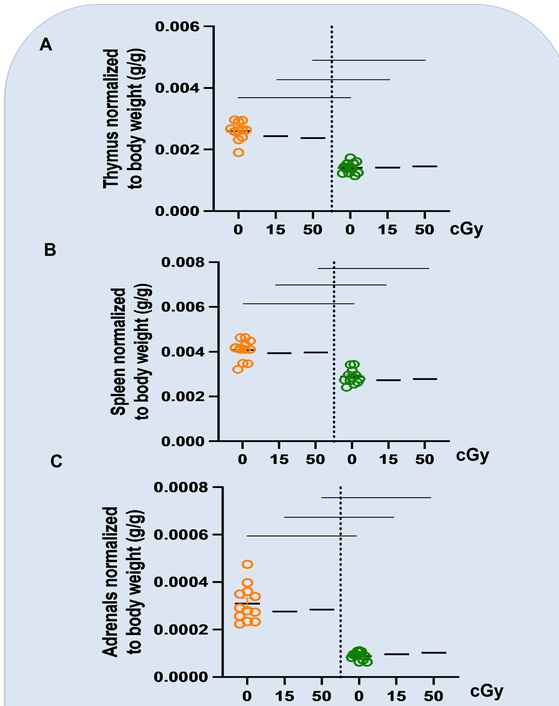


Figure 2. Immune and endocrine tissue weights at 2-weeks post-irradiation. Male and female mice were exposed to GCRsim (0, 15, and 50 cGy) and at 14-days post-IR mice were euthanized and immune thymus (A) and spleens (B) organs; and endocrine, adrenal (C) glands were isolated and weighed. All tissue weights were normalized to total body weights. Females (orange) and males (green) are displayed. Parametric or nonparametric analyses were performed with post hoc analyses as describe in the methods section. Data represent ±SEM, $p^* < 0.05$, n= 10-12 per group.

Key Findings

- Sex-, but not dose-specific immune and endocrine organ weights identified. Males displayed reduced weights across all organs.
- Sex-, but not dose-specific endocrine hormone differences identified. Aldosterone displayed reduced concentrations in males verses females.
- Differential gene expression analyses reveal unique, sex-specific profiles in response to radiation.
- *Cyp11b2* (enzyme required for aldosterone production) was downregulated in response to radiation in females, but was no different in males.
- Sex- and dose-specific effects are observed following cosmic radiation.

Future Direction

- Personalized medicine is an important consideration for spaceflight initiatives.
- To determine the impact of aldosterone on sex-specific immunity.

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