

<b>Fiscal Year:</b>	FY 2019	<b>Task Last Updated:</b>	FY 11/19/2018
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<b>Project Title:</b>	Oxidative Stress and the Cancer Risk of Space Radiation		
<b>Division Name:</b>	Human Research		
<b>Program/Discipline:</b>			
<b>Program/Discipline--Element/Subdiscipline:</b>	HUMAN RESEARCH--Radiation health		
<b>Joint Agency Name:</b>	<b>TechPort:</b>	No	
<b>Human Research Program Elements:</b>	(1) <b>SR</b> :Space Radiation		
<b>Human Research Program Risks:</b>	(1) <b>Cancer</b> :Risk of Radiation Carcinogenesis		
<b>Space Biology Element:</b>	None		
<b>Space Biology Cross-Element Discipline:</b>	None		
<b>Space Biology Special Category:</b>	None		
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<b>Project Type:</b>	Ground	<b>Solicitation / Funding Source:</b>	2013-14 HERO NNJ13ZSA002N-RADIATION
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<b>No. of PhD Candidates:</b>	1	<b>No. of Master' Degrees:</b>	0
<b>No. of Master's Candidates:</b>	1	<b>No. of Bachelor's Degrees:</b>	0
<b>No. of Bachelor's Candidates:</b>	2	<b>Monitoring Center:</b>	NASA JSC
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<b>Flight Program:</b>			
<b>Flight Assignment:</b>	NOTE: Extended to 1/14/2020 per NSSC information (Ed., 3/12/19)		
<b>Key Personnel Changes/Previous PI:</b>			
<b>COI Name (Institution):</b>	de Toledo, Sonia Ph.D. ( Rutgers University, New Jersey Medical School ) Howell, Roger Ph.D. ( Rutgers University, New Jersey Medical School ) Pain, Debkumar Ph.D. ( Rutgers University, New Jersey Medical School )		
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**Task Description:**

The objective of this project is to investigate transient and persistent oxidative stress, and its association with cancer induction, after exposure of mice to low doses/fluences of different types of space radiation. The proposal is based on the hypothesis that space radiations with different biophysical properties induce distinct redox-modulated biochemical changes. Such changes may differentially perturb physiological functions and may induce DNA damage to different extents. If they persist, some of these changes may lead to cancer. This is an immediate concern to NASA, particularly in the context of long-duration exploratory space missions. This proposal will use middle-aged mice to determine the effects of space radiation on critical redox-modulated cellular processes. Experiments will include exposures to low doses of different high energy particles (oxygen, calcium, and silicon), delivered at low dose-rate. The results will be compared with those obtained in mice exposed in parallel to cesium-137 gamma rays. We will examine acute and chronic oxidative changes in DNA, and in lipids and proteins involved in critical signaling pathways that mediate the cellular responses to stress. We will measure these changes in radiation sensitive and resistant organs following whole or partial body irradiation of mice strains that vary in their susceptibility to cancer. We will also investigate stressful effects in irradiated organs/tissues and their propagation to non-irradiated organs/tissues. We will explore the possibility that prior exposure to high energy protons induces mechanisms that protect tissues from the targeted and non-targeted stresses due to a subsequent exposure to low fluences of highly damaging energetic particles. The goal is to generate data related to Specific Gaps in knowledge listed in Cancer 1-Cancer 5 and in Cancer-7, which may help reduce the uncertainty in estimating cancer risk to astronauts.

**Rationale for HRP Directed Research:****Research Impact/Earth Benefits:**

There is overwhelming evidence to support that oxidative stress contributes to elevated levels of DNA damage, abnormal growth control, and altered metabolic pathways, which can lead to cancer. However, the effects of space ionizing radiation (IR) on these processes in vivo and the underlying signaling events have not been identified, particularly in the context of chronic exposure to low fluences of energetic high atomic number and high energy (HZE) particles that vary in their linear energy transfer (LET). The issue is further complicated by the fact that astronauts are exposed to mixed types of IR. An exposure to a low dose of low-LET IR prior to a dose from high-LET IR may induce protective processes that attenuate the damaging effects of the latter. This is important because the low flux of the high-LET HZE radiations in space relative to the higher flux of low-LET protons makes it highly probable that for any given cell in the body, proton events will precede any HZE event. Assessing these targeted and non-targeted responses will synergize with other NASA supported studies and will contribute crucial and novel mechanistic information to ongoing efforts in developing biophysical models for predicting health risks to astronauts. By achieving an integrated understanding of the endpoints investigated in this proposal, a rational path towards preventing the occurrence or delaying the onset of cancer (and other adverse health effects) during or after space missions may be developed. Further, as particle therapy is being increasingly used to treat cancer, the proposed studies may lead to the development of treatment protocols that enhance the efficacy of anti-tumor treatments and attenuate post therapeutic out-of-field normal tissue toxicity.

1. Effects of Space Radiation on Abundance of Hematopoietic Cells and the Bone Marrow Microenvironment  
Effect on the bone marrow niche assessed by computed tomography (micro-CT): In last year's progress report, we described temporal changes in the relative abundance of circulating immune cells, and of their precursors in bone marrow of control mice, and mice exposed to either energetic proton or energetic heavy particles (HZE particles). The results were compared with those obtained in mice exposed in parallel to gamma rays from a cesium-137 source (acute whole-body exposure to a dose of 150 or 300 cGy). Increases up to 10-fold in circulating immune cells (neutrophils and monocytes) were detected in mice following two weeks of exposure to 20, 30, or 40 cGy of any of the heavy ions used in the study (1 GeV/nucleon calcium, silicon, oxygen). These increases were observed whether the radiation exposure was delivered in a single bolus or in a fractionated manner. Furthermore, the increases in neutrophils and monocytes in circulating blood were associated with decreased abundance of these cell subsets in bone marrow. The common myeloid progenitors, as well as the granulocyte/macrophage, and megakaryocyte-erythroid progenitors were also significantly decreased in the bone marrow, together with significant decreases in short-term hematopoietic stem cells.

During this reporting period, we have investigated the bone marrow niche by micro-CT (high resolution computerized tomography). Prominent morphological changes in trabecular bone at the distal femur metaphysis were detected at two weeks after whole body exposure of the mice to 40 cGy of 1 GeV/n oxygen ions delivered as single acute bolus. A 3-dimensional view revealed that the trabecular bone has changed from plate-shape to rod-shape in the irradiated mice supporting an effect on the bone marrow niche where decreases in the progenitor cells were detected. Analysis of the number of nodes and struts in the trabecular bone in femur is ongoing.

Plasma cells: We have expanded our analyses on the abundance of circulating hematopoietic cells. Whereas, at early times (2 weeks – 3 months) increases in neutrophils and monocytes were significant in peripheral blood of irradiated mice, the abundance of these cells at 14 months was not different from that in control mice. However, significant increases were detected in plasma cells, which is suggestive of plasma cell proliferative diseases.

Natural killer (NK) cells: Decreased NK activity of 30% and 25% when compared to 0 Gy control, respectively, were found in spleen of mice exposed to 150 cGy gamma rays (acute) or 40 cGy from energetic oxygen ions delivered in 3 fractions as measured by the chromium release assay. The percentage of splenocytes that are NK by flow cytometry in the irradiated samples were not statistically different from those in control. Together, the results suggest that both the sparsely ionizing gamma rays (acute) and densely ionizing 40 cGy oxygen ions (3 fractions) exposures can lead to long term decreases in NK activity.

## 2. Tissue injury

Consistent with events leading to a persistence of inflammatory responses, ongoing investigation of late injury in the various harvested mouse organs is revealing significant fibrosis in lung, liver, spleen, and heart at 15 months after whole body exposure to HZE particles. This late expression of fibrosis is associated with nitrosative stress (a process associated with inflammation) as revealed by up-regulation of 3-nitrotyrosine modified proteins. Analyses of other biomarkers of inflammation/oxidative stress were also performed on lung tissue at 2 weeks and 15 months after whole body irradiation, including TGFbeta, pSMAD2, IL-6, CYP2b10, and TNFalpha. In particular, IL-6, which participates in the inflammatory process was increased in liver at 15 months after HZE-particle irradiation.

We have pursued in situ studies of telomeric DNA double strand breaks (DNA breaks at end of chromosomes, an

<p><b>Task Progress:</b></p>	<p>indicator of ageing), and analyzed inflammatory responses (reactive microgliosis and astrogliosis) in the hippocampus and striatum in brain sections of the HZE-particle-irradiated mice. We found that at 6 hours after the end of the irradiation protocol, ~2% of cells displayed few and discrete gammaH2AX foci (a marker of DNA damage) that were completely resolved by 2 weeks after irradiation. In addition, only 31% of gammaH2AX foci co-localized with telomeres, demonstrating that most DNA damage occurs in non-telomeric DNA. Surprisingly, in contrast to control animals, gammaH2AX positive cells increased with advancing age, irradiated animals did not, suggesting the possibility of radiation stimulated mechanisms that protect against age-related degenerative processes in the brain. We did not detect significant evidence of microgliosis or astrogliosis in the striatum of mice exposed to mean absorbed dose of 40 cGy of 1 GeV/nucleon calcium, silicon, or oxygen particles delivered in a fractionated manner to the whole body.</p> <p>Cardiovascular: We have completed evaluation of cardiac function by echocardiography in control mice, and in heavy ion-irradiated mice (1 GeV/nucleon calcium, silicon, or oxygen particles, 20 cGy or 40 cGy delivered in a fractionated manner) or gamma-irradiated mice (150 cGy, single acute bolus). The measurements were acquired at 15 months after the radiation exposure in 8-12 mice/group. Whereas mice exposed to energetic calcium ions showed statistically significant change in left ventricular mass following exposure to fractionated doses of 20 or 40 cGy, the mice exposed to fractionated dose of 40 cGy from energetic oxygen or silicon ions did not show significant changes for this endpoint. The mice exposed to 150 cGy of gamma rays showed a significant change in the left ventricular mass, but the change was not as large as in the calcium-irradiated mice.</p> <p>Classical bone morphogenetic proteins (BMPs) were originally named for their osteo-inductive properties. We have pursued analyses of BMP2 signaling in the aorta of control and calcium-irradiated mice. The results indicated decrease in BMP2 signaling inferred through phosphorylation of BMP2 downstream effectors SMAD1/5/9 proteins. This decrease in BMP2 signaling was associated as expected with an increase in TGFbeta (although, the TGFbeta increase did not reach statistical significance). These experiments are being expanded to investigate the effects of energetic silicon and oxygen ions.</p> <p>Bone: Exposure to densely ionizing particles may result in modulation of bone formation, resorption, and mineralization. To characterize the effects of energetic heavy (HZE) particles on bone dynamics in living mammals, a radio-densitometric evaluation of bone was conducted 15 months following exposure of the mice to fractionated doses of energetic calcium, silicon, or oxygen ions (isovelocity 1 GeV/nucleon). Relative bone density of mice from the various treatment groups was evaluated by comparing the frequency distribution of the image voxel intensities within a range of radio-density known to encompass low and high-density bone. Six to eight mice per treatment group were imaged. Intra-group comparisons showed that the bone density distributions for the mice in the 0 cGy (control) group exhibit a high degree of overlap, displaying limited variation throughout the distributions. In contrast, the data for the irradiated mice revealed marked distribution heterogeneity within these test groups. Further micro-CT imaging of Ca-irradiated mice and their respective control showed abnormalities detected in femurs of the irradiated mice, which suggests aberrant mineralization. These scanning results are being analyzed to gain quantitative information.</p> <p>In summary, our ongoing studies in mice revealed that exposure to energetic heavy particles when the mice are 10 month-old leads to both short- and long-term biological changes that can have a significant impact on health. Exposure to moderate mean absorbed doses of space radiation : i) Induces persistent oxidative stress and inflammatory effects; ii) perturbs abundance of hematopoietic cells; iii) fractionation of the dose from energetic heavy particles results in greater level of tissue damage than induced by a single bolus; iv) exposure to energetic heavy particles induces non-targeted effects; v) in contrast to our in vitro results, low dose of 1 GeV protons delivered at low dose rate does not appear to induce significant adaptive responses as measured by the endpoints analyzed so far in this study; vi) analysis of cancer induction is in progress: paraffin-embedding and sectioning of tissues is ongoing. The slides will be assessed by expert pathologists; vii) analyses of oxidative stress and inflammatory responses in non-targeted organs following head only irradiation are being initiated, together with analytical studies in mice exposed to protons prior to exposure to energetic calcium ions.</p>
<p><b>Bibliography Type:</b></p>	<p>Description: (Last Updated: 04/05/2023)</p>
<p><b>Articles in Peer-reviewed Journals</b></p>	<p>Sharma N, Moore L, Chidambaram S, Colangelo NW, de Toledo SM, Azzam EI. "c-Jun N-terminal kinase inhibition induces mitochondrial oxidative stress and decreases survival in human neural stem progenitors." Dev Neurosci. 2018 Dec;40(4):312-24. Epub 2018 Oct 18. <a href="https://doi.org/10.1159/000493009">https://doi.org/10.1159/000493009</a> ; PubMed <a href="https://pubmed.ncbi.nlm.nih.gov/30336480/">PMID: 30336480</a> , Dec-2018</p>