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Project Title:	Oxidative Stress and the Cancer Risk of Space Radiation		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Radiation health		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **SR**:Space Radiation		
Human Research Program Risks:	(1) **Cancer**:Risk of Radiation Carcinogenesis		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
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Zip Code:	07103	**Congressional District:**	10
Comments:			
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Key Personnel Changes/Previous PI:			
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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.
	Progress in accomplishing the research outlined in our investigation of oxidative stress and the cancer risk of space radiation has proceeded as planned. We have completed the irradiation of mice in March 2017, and carried out experiments related to the three Specific Aims outlined in the project. Briefly, middle-aged CBA/CaJ male mice (9-10 month old) were exposed (whole or partial body) to isovelocity 1 GeV/u protons, calcium, silicon, or oxygen ions with respective average Linear Energy Transfer (LET) values of ~0.24, 14, 44, and 88 keV/µm. A different set of mice was exposed to cesium-137 gamm rays as reference radiation (LET ~ 0.9 keV/ µm) to examine the following: 1- To assess chronic oxidative stresses, inflammatory responses, and degenerative conditions in organs that differ in their radiation sensitivity. 2- To evaluate the relative biological effectiveness of the space radiations compared to acute cesium-137 gamma rays in enhancing the rate of cancer incidence 3- To measure oxidative changes and cancer incidence in non-irradiated organs after exposure of the head to a moderate dose (0.4 Gy) of high atomic number (Z) and high energy (E) HZE particles, and to compare the observed changes with those in the targeted organ (brain). 4- To examine the protective effect of whole-body pre-exposure to a conditioning dose of 0.2 Gy of 1 GeV protons delivered at low dose-rate prior to head exposure to acute dose of 0.4 Gy of HZE particles. At 6 h, 2 weeks, and 3, 6, and 10 months after irradiation, 5 mice or more from each of the groups described below were anesthetized and peripheral blood was collected. The mice were then perfused with saline and different organs (heart, liver, lung, kidney, bone marrow, brain, reproductive organs, eyes, femurs) were harvested for cellular, biochemical, molecular, and histological analyses, and for archiving in NASA's Space Radiation Tissue Sharing Forum. At 15 months the remainder of the mice (60-150) in each group were sacrificed. At this latter time point, a set of live mice (n=6-12) was scanned by computed tomography and ultrasound-echocardiography. The groups of mice were as follows: 1: Control ; 2: Gamma rays: 1.5 Gy (acute single bolus, whole body) ; 3: Gamma rays: 3 Gy (acute single bolus, whole body) ; 4: 1 GeV protons: 0.2 Gy (0.0035 Gy/min, whole body) ; 5: 1 GeV/u Ca: 0.2 Gy (in 3 fractions; 1 acute fraction/day; whole body) ; 6: 1 GeV/u Ca: 0.3 Gy (in 3 fractions; 1 acute fraction/day; whole body) ; 7: 1 GeV/u Ca: 0.4 Gy (in 3 fractions; 1 acute fraction/day; whole body) ; 8: 1 GeV/u Ca: 0.4 Gy (acute single bolus; whole body) ; 9: 1 GeV/u Ca: 0.4 Gy (acute single bolus; head only) ; 10: 1 GeV/u Si: 0.4 Gy (in 3 fractions; 1 acute fraction/day; whole body) ; 11: 1 GeV/u Si: 0.4 Gy (acute single bolus; whole body) ; 12: 1 GeV/u O: 0.4 Gy (in 3 fractions; 1 acute fraction/day; whole body) ; 13: 1 GeV/u O: 0.4 Gy (acute single bolus; whole body) ; 14: 1 GeV protons followed by 1 GeV/u Ca: whole body exposure to 0.2 Gy of protons delivered 24 h prior to 0.4 Gy of 1 GeV/u Ca ions delivered to the whole body ; 15: 1 GeV protons followed by 1 GeV/u Ca: whole body exposure to 0.2 Gy of protons delivered 24 h prior to 0.4 Gy of 1 GeV/u Ca ions targeted to the head only. HIGHLIGHTS FROM THE RESULTS: At 2 weeks, and 3, 6, and 15 months after irradiation, peripheral blood and bone marrow were drawn from at least 5 mice of each of the groups described above to examine alterations in cell subsets using multicolor flow cytometry. Relative percent change in specific cell populations and absolute cell counts were determined. Increases up to 10-fold in circulating neutrophils were detected at two weeks in mice exposed (whole body) to 20, 30, or 40 cGy of either of the heavy ions delivered in a fractionated manner ($p<0.001$). The groups also showed an increase in circulating monocytes ($p<0.01$). The mice exposed to a single bolus of 40 cGy of Ca ions did not show significant increases at 2 weeks; however, by 3 months, increases in neutrophils were detected ($p<0.001$). These increases in neutrophils and monocytes in circulating blood were associated with decreases in these cell subsets in bone marrow ($p<0.05$), suggesting mobilization out of this compartment. Common myeloid, as well as granulocyte / macrophage and

Task Progress:	megakaryocyte-erythroid progenitors were decreased (p<0.01) in bone marrow. Notably, decreases (p<0.01) in short-term hematopoietic stem cells were detected. The alterations observed at 2 weeks were associated with changes in the levels of circulating inflammatory cytokines (e.g., TNF-alpha, IL-1 beta, CXCL1). Furthermore, histological analyses revealed prominent interstitial lung disease in mice exposed to a single bolus of 40 cGy of heavy ions, characterized with thickened alveolar septa, pulmonary congestion, and endothelial hyperplasia. The mice exposed to the fractionated regimens presented mild lung injury. The early response of neutrophils and monocytes in mice exposed to the energetic heavy ions returned to a normal range at 6 months after irradiation, and remained in this normal range at 14 months. However, at the latter time point, the proportion (%) of circulating plasma cells, but not B cells, were increased (p<0.001) in mice exposed to 30 or 40 cGy of Ca ions delivered in a fractionated manner. The effect seems to depend on the radiation dose and delivery manner, as it was not detected in mice exposed to 20 cGy of Ca ions delivered in a fractionated manner, nor in mice exposed to 40 cGy of Ca ions delivered in a single bolus. Similar finding occurred in mice exposed to 40 cGy of Si ions, but not in mice exposed to gamma rays or protons. This indicates the phenotypes may be specific to high LET radiations, and is suggestive of the development of a plasma cell dyscresia. Analyses of the long-term effects of exposure to 1 GeV/u oxygen ions will occur in March 2018. Relative to control, at 15 months after irradiation of mice with energetic Ca ions, an increase in the hematocrit percentages was detected in animals irradiated with 40 cGy delivered as a single bolus to the whole body (p = 0.03). There were massive decreases in circulating triglycerides in the blood serum after irradiation with 20 or 40 cGy delivered in a fractionated manner. There were also increases in blood glucose. There were no changes in the concentration of high density lipoproteins (HDL) and low density lipoproteins (LDL) in all groups. There was an increase in alanine transaminase (ALP) in blood upon irradiation with a fractionated dose of 20 cGy delivered to the whole body. Preliminary studies show that exposure to HZE particles leads to oxidative modification of proteins from different organs that persist over time. In addition, alterations in signaling pathways (in particular Bone Morphogenetic Protein (BMP) signaling) was detected in aorta. Proteomic studies in cerebellums of mice exposed to 40 cGy of 1 GeV/u Ca ions: We determined the global S-nitrosylation patterns (S-nitroso proteome) using `Biotin Switch' assay coupled with mass spectrometry (MS) analyses. The resulting expression patterns of proteins (general proteome) and S-nitrosylated protein (S-nitroso proteome) are being analyzed by bioinformatics classification data mining tools and pathway analysis tools. In addition, S-nitrosylation sites will be examined by computational biology and structural bioinformatics analysis tools to obtain stereochemical and physicochemical characteristics of S-nitrosylation sites in proteins. Computed tomography scans of bone: Intra-group co-plots showed that the normalized bone density distributions for the mice in the 0 cGy (control; n=6) group exhibit a high degree of overlap, displaying limited variation throughout the distributions. Interestingly, intra-group co-plots of the data for the 20 cGy Ca ion and 150 cGy gamma ray groups revealed marked distribution heterogeneity within these test groups. Cardiac function evaluated by echocardiography. Vevo2100 (VisualSonics) ultrasound equipment was used to assess echocardiographic endpoints in sham-irradiated mice or mice exposed to 40 cGy of 1 GeV/u 40Ca ions delivered in 3 fractions over 3 days or in mice exposed to 150 cGy acute 137Cs gamma rays (n = 6-9/each group). Several parameters were assessed. Preliminary analyses revealed that exposure to energetic Ca ions results in significant effects on stroke volume, cardiac output, and the left ventricular mass index. This work is currently proceeding with larger sample numbers and in mice exposed to protons, silicon and oxygen ions. Gross pathological changes: At 15 months after irradiation, preliminary studies indicate significant abnormalities (p<0.01) in seminal vesicle and orbital tissues of mice exposed 15 months earlier to energetic calcium ions delivered to the whole body or head only.
Bibliography Type:	Description: (Last Updated: 04/05/2023)
Abstracts for Journals and Proceedings	Moore L, Guo J, Chidambaram S, Hu T, de Toledo SM, Azzam EI. "Degenerative effects in tissues of CBA/CaJ mice exposed to Space Radiations." Presented at the 63rd Annual Meeting of the Radiation Research Society, Grand Fiesta Americana Coral Beach, Cancun, Mexico, October 14-18, 2017. 63rd Annual Meeting of the Radiation Research Society, Grand Fiesta Americana Coral Beach, Cancun, Mexico, October 14-18, 2017. Abstract PS 3-38. , Oct-2017
Articles in Peer-reviewed Journals	de Toledo SM, Buonanno M, Harris AL, Azzam EI. "Genomic instability induced in distant progeny of bystander cells depends on the connexins expressed in the irradiated cells." International Journal of Radiation Biology. 2017 Oct;93(10):1182-94. Epub 2017 Jun 15. https://doi.org/10.1080/09553002.2017.1334980 ; PubMed PMID: 28565963 , Oct-2017
Articles in Peer-reviewed Journals	Baljinnyam E, Venkatesh S, Gordan R, Mareedu S, Zhang J, Xie LH, Azzam EI, Suzuki CK, Fraidenraich D. "Effect of densely ionizing radiation on cardiomyocyte differentiation from human-induced pluripotent stem cells." Physiological Reports. 2017 Aug;5(15):e13308. https://doi.org/10.14814/phy2.13308 ; PubMed PMID: 28801517; PubMed Central PMCID: PMC5555881 , Aug-2017
Books/Book Chapters	Zhang J, Shim G, de Toledo SM, Azzam EI. "The translationally controlled tumor protein and the cellular response to ionizing radiation-induced DNA damage." in "TCTP/tpt1 - Remodeling Signaling from Stem Cell to Disease." Ed. A. Telerman, R. Amson. Cham: Springer International Publishing AG, 2017. Chapter 12, p. 227-253. https://doi.org/10.1007/978-3-319-67591-6_12 [Ed. note: also shows in PubMed, PubMed PMID: 29149412] , Nov-2017