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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		
Space Biology Special Category:	None		
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Comments:			
Project Type:	Ground	Solicitation / Funding Source:	2013-14 HERO NNJ13ZSA002N-RADIATION
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No. of PhD Candidates:	2	No. of Master' Degrees:	1
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No. of Bachelor's Candidates:	0	Monitoring Center:	NASA JSC
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Flight Program:			
Flight Assignment:			
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. Our team has participated in three NASA Space Radiation Laboratory (NSRL) runs during 2016 (Runs 16A, 16B, and 16C). With outstanding support from the concerned staff at the Brookhaven National Laboratory, the experiments were conducted successfully. In studies related to the three Specific Aims outlined in the project, groups of middle-aged CBA/CaJ mice (9-10 month old) were exposed (whole body) during run 16A to either 1 GeV protons, 1 GeV/u calcium (Ca) ions, or cesium-137 gamma rays. During run 16B they were exposed to 1 GeV/u silicon (Si) ions or cesium-137 gamma rays, and during run 16C they were exposed to 1 GeV/u oxygen (O) ions to examine the following:

- 1- Chronic oxidative stresses and inflammatory responses in organs that differ in their radiation sensitivity following exposure of the mice to the different radiation types described above;
- 2- To evaluate the relative biological effectiveness of isovelocity protons and high atomic number and high energy (HZE) particles described above 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 (liver, lung) after exposure of the head to an acute mean absorbed dose of 0.4 Gy of 1 GeV/u Ca, 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 1 GeV/u Ca ions.

At two weeks, and 3, 6, and 10 months after irradiation, 5 mice from each of the groups described below were sacrificed, and peripheral blood as well as different organs (heart, liver, lung, kidney, bone marrow, brain, and testes) were harvested for cellular, biochemical, molecular, and histological analyses, as well as for archiving and participation in NASA's Space Radiation Tissue Sharing Forum. The groups of mice were as follows:

1: Control; 2: gamma rays: 1.5 Gy (acute, single fraction, whole body); 3: gamma rays: 3 Gy (acute, single fraction, 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 (in 1 acute fraction; whole body); 9: 1 GeV/u Ca: 0.4 Gy (in 1 acute fraction; 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 (in 1 acute single fraction; 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 (in 1 acute single fraction; 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.

Task Progress:

PRELIMINARY RESULTS: Here we report analyses performed in unirradiated control mice and in mice exposed to either 1 GeV protons, 1 GeV/u Ca ions, or cesium-137 gamma rays 2 weeks, and 3 months earlier. We examined alterations in circulating hematopoietic cell subsets using multicolor flow cytometry. The relative percent change in specific cell populations and absolute cell counts were also determined. Significant changes were detected in cell subsets after exposure to the energetic Ca ions. Specifically, ~ 10-fold increase in neutrophils were detected at two weeks in mice exposed to 20, 30, or 40 cGy of Ca ions delivered in a fractionated manner. The groups also showed

	<p>significant increase in macrophages. These increases were attenuated by 3 months. 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. The alterations observed at 2 weeks were associated with changes in the levels of circulating inflammatory cytokines (e.g., TNF-alpha, IL-1beta, CXCL1). Furthermore, histological analyses revealed prominent interstitial lung disease in mice exposed to a single bolus of 40 cGy of Ca ions at 2 weeks, characterized with thickened alveolar septa, pulmonary congestion, and endothelial hyperplasia. The mice exposed to the fractionated regimens presented mild lung injury.</p> <p>In addition to the above, our preliminary studies show that 1 GeV/u Ca ions (20 cGy administered in 3 acute fractions over 3 days), and protons (20 cGy administered in a single bolus delivered over 60 min), lead to oxidative modification of cardiac proteins detectable by Oxyblot analysis two weeks after irradiation. Analyses of oxidative stress in other tissues (liver, brain) are ongoing.</p> <p>Mass spectrometry (MS) and statistical analyses to examine the effects of low and moderate mean absorbed doses of HZE particles on the modulation of SNO protein levels in mice brains two weeks following whole body irradiation with 0.5 GeV/u titanium ions performed in earlier studies are nearing completion. Protein S-nitrosylation (SNO) is a reversible post-translational modification (PTM) through the covalent addition of nitric oxide (•NO) onto cysteine thiols. Strikingly, compared to control, greater increases in global S-nitrosylation of brain proteins were detected after exposure to low (5 cGy) than moderate (50 cGy) doses. Immunoprecipitation of candidate proteins (e.g., peroxiredoxin or Prx-1) confirmed the mass spectrometry results. Furthermore, Western blot analyses revealed upregulation of inducible nitric oxide synthase (iNOS) levels in brains exposed to 5 cGy. Notably, Prx-1 and iNOS are implicated in regulation of numerous oxidative stress-responsive pathways. Studies of protein nitrosylation in the cerebellum of mice at 2 weeks after exposure to 0 or 40 cGy of 1 GeV/u Ca ions delivered in a single bolus to the whole body or to the head only are ongoing.</p>
Bibliography Type:	Description: (Last Updated: 04/05/2023)
Abstracts for Journals and Proceedings	<p>Hu T, Colangelo NW, Domogauer JD, Schwartzkopf C, Howell RW, de Toledo SM, Aisner S, Azzam EI. "Exposure of mice to space radiation modulates the abundance of hematopoietic cells and inflammatory cytokines." Presented at the 62nd Annual Meeting of the Radiation Research Society, Big Island, Hawaii, October 16-19, 2016.</p> <p>62nd Annual Meeting of the Radiation Research Society, Big Island, Hawaii, October 16-19, 2016. Abstract book, PSS-67. , Oct-2016</p>
Abstracts for Journals and Proceedings	<p>Pan S, Shibata M, Azzam EI. "Analyses of proteomic alterations in brains of mice exposed to space radiation." Presented at the concluding Symposium of the "Interdisciplinary Training Program in Cancer Research," RUTGERS Biomedical and Health Sciences, New Jersey Medical School, Newark, NJ, August 2, 2016.</p> <p>Proceedings of Cancer Summer Projects. From concluding Symposium of the "Interdisciplinary Training Program in Cancer Research," RUTGERS Biomedical and Health Sciences, New Jersey Medical School, Newark, NJ, August 2, 2016. , Aug-2016</p>
Articles in Peer-reviewed Journals	<p>Norbury JW, Schimmerling W, Slaba TC, Azzam E, Badavi FF, Baiocco G, Benton E, Bindi V, Blakely EA, Blattnig SR, Boothman DA, Borak TB, Britten RA, Curtis S, Dingfelder M, Durante M, Dynan W, Eisch AJ, Robin Elgart S, Goodhead DT, Guida PM, Heilbronn LH, Hellweg CE, Huff JL, Kronenberg A, La Tessa C, Lowenstein D, Miller J, Morita T, Narici L, Nelson GA, Norman RB, Ottolenghi A, Patel ZS, Reitz G, Rusek A, Schreurs A-S, Scott-Carnell LA, Semones E, Shay JW, Shurshakov VA, Sihver L, Simonsen LC, Story M, Turker MS, Uchihori Y, Williams J, Zeitlin CJ. "Galactic cosmic ray simulation at the NASA Space Radiation Laboratory." Life Sciences in Space Research 2016 Feb;8:38-51. http://dx.doi.org/10.1016/j.lssr.2016.02.001 ; PMID: 26948012 , Feb-2016</p>
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Articles in Peer-reviewed Journals	<p>Domogauer JD, de Toledo SM, Azzam EI. "A mimic of the tumor microenvironment: A simple method for generating enriched cell populations and investigating intercellular communication." J Vis Exp. 2016 Sep 20;(115):e54429. http://dx.doi.org/10.3791/54429 ; includes video component: http://www.jove.com/video/54429 ; PMID: 27684198 , Sep-2016</p>