Fiscal Year:	FY 2022	**Task Last Updated:**	FY 01/29/2022
PI Name:	Shay, Jerry W. Ph.D.		
Project Title:	Mouse Models of Cancer Risk and Prevention from 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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Zip Code:	75390-7208	**Congressional District:**	30
Comments:			
Project Type:	GROUND	**Solicitation / Funding Source:**	2014-15 HERO NNJ14ZSA001N-RADIATION. Appendix D: Ground-Based Studies in Space Radiobiology
Start Date:	01/29/2016	**End Date:**	09/30/2022
No. of Post Docs:	2	**No. of PhD Degrees:**	1
No. of PhD Candidates:		**No. of Master' Degrees:**	
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No. of Bachelor's Candidates:		**Monitoring Center:**	NASA JSC
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Flight Program:			
Flight Assignment:	NOTE: End date changed to 9/30/2022 per L. Barnes-Moten/JSC (Ed., 3/30/21) NOTE: End date changed to 1/28/2021 per NSSC information (Ed., 2/21/2020)		
Key Personnel Changes/Previous PI:	November 2020 report: Silvia Siteni, PhD postdoctoral trainee; Krishna Luitel (completed PhD) now a postdoctoral trainee.		
COI Name (Institution):			
Grant/Contract No.:	NNX16AE08G		
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Task Description:	Overall hypothesis: Low-dose radiation induces molecular manifestations of a pro-inflammatory response as a function of radiation type, radiation doses, doses rates, LET (linear energy transfer) value, and time. An oral available anti-inflammatory countermeasure, already in human clinical trials with a good safety profile, will significantly reduce proton and HZE-ion (high charge energy-ion) exposure associated tumor initiation and progression. The overarching hypothesis for this project is that space radiation induces molecular manifestations of a pro-inflammatory response as a function of radiation type, radiation doses, doses rates, LET value, and time. We are testing if an oral available anti-oxidant and anti-inflammatory countermeasure, already in human clinical trials with a good safety profile, CDDO, significantly reduces proton and HZE-ion exposure associated tumor initiation and progression. Based on experiments conducted at the NASA Space Radiation Laboratory (Brookhaven, NY) we demonstrate that HZE ion components of GCR (galactic cosmic radiation) result in persistent DNA damage and inflammatory signaling, increased mutations in tumor suppressor genes, and higher rates of cancer initiation and progression compared to that seen with similar doses of terrestrial radiation. While physical shielding may reduce some of the risks of space radiation, there is substantial evidence that biological countermeasures will be required to ensure that the established safety limits of increased lifetime fatal cancer risks are not exceeded. We are conducting GCR simulations consisting of fast switching between protons, helium, and silicon using a dose rate of 0.5 cGy/min and a total combined dose of between 27-30 cGy to more closely mimic the space environment on a trip to Mars and back. Finally, we are conducting experiments with the official NASA GCRsim with acute and protracted mixed fields. References Kim, S.B., Bozeman, R.G., Kaisani, A., Kim, W., Zhang, L., Richardson, J.A., Wright, W.E., and Shay, J.W. Radiation promotes colorectal cancer initiation and progression by inducing senescence-associated inflammatory responses. Oncogene. 2015. https:// Norbury, J.W., Schimmerling, W., Slaba, T.C., Edouard Azzam, Francis F. Badavi, Giorgio Baiocco, Eric Benton, Veronica Bindi, Eleanor A. Blakely, Steve R. Blattnig, David A. Boothman, Thomas B. Borak, Richard A. Britten, Stan Curtis, Michael Dingfelder, Marco Durante, William Dynan, Amelia Eisch, S. Robin Elgart, Dudley T. Goodhead, Peter M. Guida, Lawrence H. Heilbronn, Christine E. Hellweg, Janice L. Huff, Amy Kronenberg, Chiara La Tessa, Derek Lowenstein, Jack Miller, Taksahi Morita, Livio Narici, Gregory A. Nelson, Ryan B. Norman, Takeo Ohnishi, Andrea Ottolenghi, Zarana S. Patel, Guenther Reitz, Adam Rusek, Ann-Sofie Schreurs, Lisa A. Scott-Carnell, Edward Semones, Jerry W. Shay, Vyacheslav A. Shurshakov, Lembit Sihver, Lisa C. Simonsen, Michael Story, Mitchell S. Turker, Yukio Uchihori, Jacqueline Williams, Cary J. Zeitlin. Galactic cosmic ray simulation at the NASA Space Radiation Laboratory. Life Sciences in Space Research 8:38-51, 2016. PMID: 26948012 Lutiel, K. Bozeman, R., Kaisani, A. Kim, S.B., Barron, S., Richardson, J.A., Shay, J.W. Proton radiation-induced cancer progression. Life Sciences in Space Research, 2018. https:// Luitel, K., Kim, S.B., Barron, S. Richardson, J.A. and Shay, J.W. Lung cancer progression using fast switching multiple ion beam irradiation and countermeasure prevention, Life Sciences in Space Research, 2019. https://
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Although radiation therapy is commonly used for treatment of many human diseases, including cancer, ionizing radiation produces reactive oxygen species that can damage both cancer and healthy cells in tissues. We have demonstrated using the biological countermeasure, CDDO, an anti-oxidant, anti-inflammatory modulator with a known mechanism of action, a reduction in both cancer initiation and progression in mouse models after exposure to either terrestrial or space radiation. We also demonstrated that CDDO can be used as a radioprotector in normal non cancerous human lung and breast epithelial cells exposed to space and terrestrial irradiations while cancer cells were not protected. This suggests the use of this oral available, non-toxic class of drug can protect non-cancerous healthy cells during radiotherapy, resulting in better outcomes with less toxicity for patients. CDDO is currently in a phase 3 clinical trial for patients with pulmonary arterial hypertension and Alport's syndrome. In preliminary experiments aspirin did not provide this protection. Other potential countermeasures such as metformin continue to be tested and show radioprotective activity. In the future, CDDO or metformin may be used to protect astronauts on long-term mission to Mars as well as patients receiving radiotherapy on Earth. These radioprotectors may also have utility in protecting first responders to nuclear accidents.
	Previously our work conducted at NASA Space Radiation Laboratory (Brookhaven, NY) demonstrated that HZE ion components of the galactic cosmic radiation (GCR) result in persistent inflammatory signaling, increased mutations, and higher rates of cancer initiation and progression compared to that seen with terrestrial radiation. Most previous charged particle radiation studies have been performed using mono-energetic single ion radiation exposures, but the deep space environment is composed of multiple ions with a wide range of energies. Consequently, we also exposed lung cancer susceptible mouse models (K-rasLA-1) at the NSRL with fast switching three ion beams: Proton (H) (120 MeV/n) 20cGy, Helium (He) (250 MeV/n) 5 cGy, and Silicon (Si) (300MeV/n) 5cGy with a dose rate of 0.5 cGy/min. In this study, we observed an increase in the incidence of lung cancer initiation and progression. Additionally, when we titrated the dose of HZE ion in the above irradiation protocol, we observed a dose-dependent effect of silicon ions delivered and observed reducing the total dose of silicon from 5 cGy, to 2 cGy and 0.5 cGy, progressively reduced cancer progression back to the background rates. Experiments can now be conducted to more closely simulate the deep space environment with the high energy and control upgrades at the NASA Space Radiation Laboratory (Brookhaven, NY). The delivery dose consists of 33 ions and an energy mix approximating the deep space environment. Using these upgrades, we performed acute exposure experiments lasting 1-2 hours, more prolonged exposure experiments lasting 10-15 hours, and chronic exposure experiments up to 4-6 weeks with a total dose of 50cGy and 75cGy. We also performed acute exposure experiments lasting 1-2 hours for 25cGy and 100cGy. We obtained histological samples from a subset of mice 100 days post-irradiation, and the remainder of the mice maintained for overall survival ending 1-year post-irradiation. With the acute exposure of 25cGy, we did not see any increase in adenocarcinoma nor a decrease in medial survival days. With the acute exposure of 100cGy, we did see a two-fold increase in the adenocarcinoma and a decrease in the median survival. When we compared acute exposures (1-2 hrs.), prolonged exposures (10-15 hrs.), and chronic exposure (26 weeks), we found a non-statistically significant trend in the increase of adenocarcinoma respectively for a dose of 50cGy and 75cGy total dose. Surprisingly, when we compared the overall survival of acute and chronic exposure for 50cGy and 75cGy total dose, we found higher median survival days with chronic exposure while acute exposure has lower

Task Progress:	median survival compared to unirradiated controls. These initial results can be interpreted to suggest carcinogenesis risks are reduced when the NASA official 33 beam GCR simulations are provided at low dose rates compared to high dose rates. Metformin is a biguanide compound used in the treatment of type 2 diabetes mellitus, showing very low cytotoxic effects, that was FDA-approved in 1995. Metformin decreases oxidative stress and DNA damage in vitro and in vivo, resulting in decreased chronic inflammation. Metformin acts mainly through the phosphorylation of adenosine monophosphate-activated protein kinase (AMPK), which has pleiotropic effects on cell metabolism. Furthermore, metformin targets mitochondria, inhibiting complex I of the electron transport chain (ETC), but the mechanisms underlying this process have not been completely elucidated. Because of its antioxidant effects, we investigated the role of metformin as a radioprotective compound. One single dose of metformin (2.5 mM) on human fibroblasts (BJs), shows an increase in the expression of phosphorylated AMPK alpha subunit and of superoxide dismutase 1 (SOD1). SOD1 acts as a transcriptional factor, protecting against oxidative DNA damage and its overexpression is associated with radioresistance in human glioma cells. Metformin decreases basal DNA damage (phosphorylation of H2AX at Ser 139 foci), and reactive oxygen species (ROS) production, and mitochondrial membrane depolarization (TMRE assay). To evaluate the radioprotective effect of metformin, cells were treated one time and irradiated 72 hours later, with 2, 4, and 6 Gy doses of gamma-rays. Cells were seeded at low density (200-1000 cells) and a colony formation assay was analyzed after 21 days. Metformin showed an increase in the surviving fraction of cells compared to the irradiated controls. Next, we investigated the radioprotective effect of metformin in vivo. Wild type 129/Sv mice were injected once per day with metformin 200mg/kg, for three consecutive days prior to exposure of 7.5 Gy of X-rays and sacrificed after 24 hours. Metformin pre-treatment was able to dramatically decrease DNA damage (p53 binding protein 1 foci) in mouse lung and colon tissues as well as the number of micronuclei in bone marrow cells, compared to the irradiated controls. Notably, when mice were irradiated at the dose of 10 Gy X-rays post-metformin treatment, a 30% increase in the surviving fraction was observed. In another experiment, wild type mice were pre-treated with metformin and irradiated with 2Gy dose of X-rays and sacrificed after 100 days. We found that metformin pre-treatment induces an increase of the expression of phosphorylated nuclear factor kappa B subunit p65 (NF-KB p65) serine536. The NF-KB p65 s536 inhibits NF-KB signaling to prevent deleterious inflammation. Next, wild type mice were irradiated with 75cGy simulated-GCR, after 72 hours of metformin treatment. Metformin 72 hours pre-treatment significantly decreased the number of micronuclei in murine bone marrow cells compared to 75cGy control GCR-sim irradiated mice. In addition, persistent oxidative stress induced by 75cGy GCR-sim in lung and colon tissues was decreased. Finally, metformin pre-administration acts as a radioprotector through AMPK phosphorylation, increasing OGG1 and decreasing cleaved Poly (ADP-ribose) polymerase (PARP) expression. All together, we interpret these results to suggest that metformin is a potential GCR radioprotector, and has the potential to lower the risk of cancer initiation/promotion in astronauts.
Bibliography Type:	Description: (Last Updated: 02/21/2024)
Articles in Peer-reviewed Journals	Kiffer FC, Luitel K, Tran FH, Patel RA, Guzman CS, Soler I, Xiao R, Shay JW, Yun S, Eisch AJ. "Effects of a 33-ion sequential beam galactic cosmic ray analog on male mouse behavior and evaluation of CDDO-EA as a radiation countermeasure." Behav Brain Res. 2022 Feb 15;419:113677. https://doi.org/10.1016/j.bbr.2021.113677 ; PMID: 34818568 , Feb-2022