Fiscal Year:	FY 2021	**Task Last Updated:**	FY 12/09/2020
PI Name:	Edwards, John Ph.D.		
Project Title:	Countermeasures to Radiation Induced Cardiomyopathy		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
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		
PI Email:	j_edwards@nymc.edu	**Fax:**	FY
PI Organization Type:	UNIVERSITY	**Phone:**	914-594-4166
Organization Name:	New York Medical College		
PI Address 1:	Department of Physiology		
PI Address 2:	15 Dana Rd		
PI Web Page:			
City:	Valhalla	**State:**	NY
Zip Code:	10595-1554	**Congressional District:**	17
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2017-2018 HERO 80JSC017N0001-BPBA Topics in Biological, Physiological, and Behavioral Adaptations to Spaceflight. Appendix C
Start Date:	02/01/2019	**End Date:**	01/31/2022
No. of Post Docs:		**No. of PhD Degrees:**	
No. of PhD Candidates:		**No. of Master' Degrees:**	1
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No. of Bachelor's Candidates:		**Monitoring Center:**	NASA JSC
Contact Monitor:	Zawaski, Janice	**Contact Phone:**	
Contact Email:	janice.zawaski@nasa.gov		
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Flight Assignment:	NOTE: End date changed to 1/31/2022 per NSSC information (Ed., 8/12/21)		
Key Personnel Changes/Previous PI:	December 2020 report: No Changes.		
COI Name (Institution):	Eisenberg, Carol Ph.D. (New York Medical College) Rota, Marcello Ph.D. (New York Medical College)		
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Task Description:	The present application seeks to study the consequences of galactic cosmic radiation (GCR) exposure. Space travel increases solar and cosmic particle radiation exposure which is significantly elevated once travel moves beyond low Earth orbit. This includes a combination of high-energy protons and heavy ions such as Fe56, Si28, and O16. Radiation induced cardiomyopathies are observed months or years after exposure. Our preliminary findings observed that GCR induced degradation of cardiac function with a phenotype that was similar to that observed following doxorubicin treatment [1]. Although there are significant differences from GCR, survivors of cancer that have undergone low-LET (linear energy transfer) radiotherapy are also at risk for several adverse health outcomes including abnormal pulmonary function, endocrine disorders, neurocognitive impairment, and heart failure [2, 3]. All these organ systems are characterized by a low turnover of cells and it is possible that an accelerated cell death and/or the failure of regeneration by progenitor cells may be the underlying cause of organ failure. Although this project initially focused on protection from cardiomyopathies, our findings has implications across all organ systems. This project has focused developing countermeasures to GCR using small molecules from a FDA (Food & Drug Administration) approved library, as well as additional molecules identified by NASA personnel as high priority compounds. These drugs are part of other ongoing investigations and their inclusion will be useful in making comparisons across platforms. With regard to the Map to Human Research, this project primarily addresses two Risks. All are designated as High LxC for longer endurance missions or long-term health and wellbeing. Countermeasures fall into three categories: Radio protectors are given prophylactically or concurrently to prevent damage. Radiation therapeutics are those that stimulate repair or regeneration processes. Radionuclide eliminators discorporate or block absorption of internalized radionuclides. This project will focus on radio protectors and radiation therapeutics with the hope of developing protocols that will diminish the need for radiation therapeutics. Drug screening will be performed on two levels; 1) broad screening using analysis that allows for high throughput, and 2) focused analysis of high value target molecules. The hierarchy for success will be: 1) ability to protect cellular function, 2) ability to protect mitochondrial function, 3) ability to ameliorate radiation induced senescence, 4) ability to protect DNA. An FDA-approved drug library from MedChem Express was chosen over other commercially library because it includes a larger number drugs directed towards DNA damage, anti-inflammation. The complete FDA approved library available from MedChem Express of more than 1500 drugs was reduced to 725 drugs, by selecting against drugs that were antibiotics or anti-parasitic. Priority was given to drugs that indicated role for DNA repair, were anti-oxidant, or anti-inflammatory. References [1] Mitry MA, Laurent D, Keith BL, Sira E, Eisenberg CA, Eisenberg LM, Joshi S, Gupte S, Edwards JG: Accelerated cardiomyocyte senescence contributes to late-onset doxorubicin-induced cardiotoxicity. Am J Physiol Cell Physiol 2020, 318:C380-C91. [2] Hudson MM, Ness KK, Gurney JG, Mulrooney DA, Chemaitilly W, Krull KR, Green DM, Armstrong GT, Nottage KA, Jones KE, Sklar CA, Srivastava DK, Robison LL: Clinical ascertainment of health outcomes among adults treated for childhood cancer. Jama 2013, 309:2371-81. [3] Dekkers IA, Blijdorp K, Cransberg K, Pluijm SM, Pieters R, Neggers SJ, van den Heuvel-Eibrink MM: Long-term nephrotoxicity in adult survivors of childhood cancer. Clin J Am Soc Nephrol 2013, 8:922-9.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	The focus of this NASA funded research project is to develop countermeasures to cosmic radiation exposure with the goal of protecting flight crews on long duration missions. However, the findings of this project will also benefit those with more Earth-bound problems. We know that airline pilots and flight attendants have a small but significantly higher risk of cancer that is directly attributable to the chronic exposure to cosmic radiation during their careers. Radiation therapy has been used for the treatment of cancer for many years, and it has long been known that these survivors are at risk for other illnesses related to their treatment. Proton Therapy is an increasingly popular radiation protocol for cancer treatments. This protocol generates similar types of radiation and energy levels that are part of the solar radiation spectrum. And unfortunately, we live in an age when terrorists might eventually gain access to weapons that will generate very high radiation exposures. Hopefully this won't happen but the lessons learned from the present investigation will have overlap to the nuclear countermeasures that others are studying. The results from the current project will hopefully contribute knowledge base that other fields will find useful.
	Our approach has utilized a high throughput screen of small molecules from an FDA approved drug library. We have as a partial bias, degradation of mitochondrial function as one readout. Several studies using RNA-seq and other "omics" analyses have repeated identified mitochondrial dysfunction as participating in the response to high-LET radiation. Mitochondrial dysfunction is also one antecedent event in the transition from a healthy to cancerous cell and in part underlies the Warburg effect, a hallmark of cancer cells. Related to this, radiation-induced cardiac dysfunction leading to heart failure remains a significant clinical problem. The heart is almost completely reliant on aerobic metabolism and healthy mitochondria are critical for cardiomyocyte function. Some of our preliminary studies have demonstrated degradation of cardiac function and mtDNA integrity as a delayed consequence of low dose 56Fe (50 cGy) radiation exposure. We have participated in three campaigns at the NASA Space Radiation Laboratory (NSRL: 19B, 19C, & 20C). All protocols to date have use cultured cells including H9c2 (myoblasts), RBL-2H3 (mast cell), Hy926 (endothelial), and ES-D3 (stem/pluripotent cell). For all campaigns, cells were exposed to a total of 75 cGy using the simplified 5-ion GCR protocol developed by NASA for use at NSRL. A follow-on paradigm was used, where the drugs were introduced shortly after GCR exposure. Run 19B included one pass through the library using 10 microM of each drug and included tests for mitochondrial function, cellular senescence, and anti-oxidant capacity. Of 725 drugs, more than 150 showed some improvement over the untreated GCR exposed cells, while more than 500 showed degradation or were ineffective. A composite score was derived for each drug and the top 160 prioritized for further testing. In the broadest terms, what we learned from 19B was that drugs used in chemotherapy, the treatment of HIV infections, as well as the antifungal drugs were not useful and likely detrimental. The "statins" generated ambiguous results.

Task Progress:	Within the160 drugs, 33 are known anti-inflammatories, while some others were also thought to have a lesser capacity as anti-inflammatory. The library included 54 COX inhibitors. Of the 7 deemed effective all were COX2 inhibitors and none were COX1 specific. 12 of 25 angiotensin converting enzyme inhibitors or AT1 antagonists were observed to be effective, while no AT2 antagonists appeared useful. The library contained 20 drugs that bound to adrenergic receptors of which 5 were deemed effective, although given their direct impact on blood pressure they are not likely to be useful as countermeasures. A common theme among the effective drugs was that they interacted with GPCR/G coupled proteins, a class of signaling proteins that mediate and control cellular function. Distinct from this are the 5-HT3 antagonists considered useful. The 5-HT3 receptors are Ca+2 activated small K+ channels. Most of the other 5-HT3 antagonists that were not deemed effective were those that bound to other 5-HT receptor isoforms or also bound to other serotonergic receptors. Clinically the 5-HT3 antagonists are used for the treatment of nausea and vomiting and are currently serving in that capacity aboard the International Space Station (ISS). Different classes of anti-diabetic medications were also observed to be useful. These included the sulfonylurea class (i.e., sulfacarbamide and glimepride) as well as repaglinide and all of these drugs are insulin secretagogues that act to stimulate insulin release from the pancreas. A common element may be their impact in intracellular Ca+2 levels, an impact that may be similar to the 5-HT3 class. Metformin was also identified as being useful and its typical role is to suppress glucose release from the liver postprandial. We also found that three of the dipeptidyl peptidase DDP-4 inhibitors (i.e., anagliptin) improved cellular function follow GCR exposure. Potentially with respect to their effect in the H9c2 cells following exposure to GCR, the efficacy of the DPP4 inhibitors may be linked to their anti-inflammatory effects mediated by the NF κ B pathway. Although the efficacies of anti-diabetic drugs in vivo are understood, it is not clear what the underlying mechanism(s) of protection might have been in response to the GCR. These experiments all utilized a follow-on paradigm that examined the restorative capability of a candidate drug when presented shortly following exposure to GCR. To determine if the delayed presentation of protective drugs impacted on recovery, cells exposed to GCRsim or control conditions were maintained in culture for one month before treatment was undertaken. Although most drugs were without effect, a few were still protective even when treatment was delayed. This included metformin and sulfacarbamide, both antidiabetic therapeutics as well as dolasetron. Using a pretreatment protocol, where the drugs were added the day prior to GCR exposure and the cells maintained in culture for a week, the results observed were similar to the follow-on protocols. Metformin, sulfacarbamide, and olmesartan offered the highest level of protection, while several others also demonstrated some protective efficacy. As with most labs throughout the country the COVID19 crisis shut the lab down. This included cancellation of NSRL 20A & 20B. We were operating at about 2% for two months (03/20-05/20) and then at about 30-40% for the next three (06/20-09/20/2020). Currently we are still under restricted access which has hampered training new students, as well as bringing in personnel to perform onsite maintenance and repairs of equipment. Separate from examining mitochondrial and cellular dysfunction as readouts, we have developed a novel cell line using an Afp-tdTomato construct, as a biomarker for the transition to a cancerous cell. Validation protocols using either ethidium bromide (0.4 µg/ml) treatment and to a lesser extent UVC light (4 j/m2) exposure significantly increased Afp-tdTomato expression. During the 20C campaign we were able to begin to test these cells. GCRsim (75 cGy) significantly increased tdTomato expression compared to the No-Radiation control cells. To date only a select group of drugs from among those promoted from previous studies have been tested. Of those dimethyl fumarate, sulfacarbamide, and pargyline all appear to be promising leads. Pargyline is an monoamine oxidase (MAO) inhibitor is in part an anti-oxidant but in vivo promotes insulin release and enhanced glucose uptake. Using a pretreatment paradigm we observed that although most drugs were without effect, some did partially block GCR induced increases in tdTomato expression. Of note sulfacarbamide partially ameliorated GCR induced increases in Afp-tdTomato expression. We had developed a LRPCR protocol to demonstrate mtDNA damage in the diabetic heart. We have just begun to apply the approach to determine DNA damage following GCR as a readout for the efficacy of candidate drugs. One hour following GCRsim (75 cGy) exposure, mtDNA damage was significantly increased compared to the No Rad control group. Cultured cells allow for testing under highly controlled conditions. The use of cultured cells is a cost-effective means for decreasing the timelines of drug discovery as well as reducing the number of animals needed for downstream stream testing. Using relatively pure cell types will allow us to begin to differentiate tissue specific responses to different protection protocols. However, this approach may also be a limitation, in that using a single cell type may miss interactions important at the tissue level. For example, in response to some stresses, mast cells of the heart release histamine and cytokines that are likely to be responsible for accelerated senescence of nearby cells. Collectively our findings are consistent with previous observations in that drugs that modulate inflammation and anti-oxidant pathways are likely to be useful. Uniquely we have observed that some 5-HT3 antagonists and some drugs used clinically in the management of diabetes appear to be useful when tested across different testing platforms, but their underlying mechanisms is not apparent.
Bibliography Type:	Description: (Last Updated: 07/05/2023)
Abstracts for Journals and Proceedings	Weiss M, Tefft K, Nikisher B, Katsett A, Edwards JG. "Countermeasures to Radiation Induced Cellular Dysfunction." 2020 NASA Human Research Program Investigators' Workshop, Galveston, TX, January 27-30, 2020. Abstracts. 2020 NASA Human Research Program Investigators' Workshop, Galveston, TX, January 27-30, 2020. , Jan-2020
Abstracts for Journals and Proceedings	Edwards JG, Weiss M, Tefft K, Nikisher B, Katsett A. "Countermeasures to the impact of cosmic radiation exposure on myocardial mitochondrial function." Experimental Biology 2020, San Diego, CA, April 4-7, 2020. Virtual Only. FASEB Journal. 2020 Apr;34(1Suppl). https://doi.org/10.1096/fasebj.2020.34.s1.06568 , Apr-2020
Articles in Peer-reviewed Journals	Mitry MA, Laurent D, Keith BL, Sira E, Eisenberg CA, Eisenberg LM, Joshi S, Gupte S, Edwards JG. "Accelerated cardiomyocyte senescence contributes to late-onset doxorubicin-induced cardiotoxicity." Am J Physiol Cell Physiol. 2020 Feb 1;318(2):C380-C391. https://doi.org/10.1152/ajpcell.00073.2019 ; PMID: 31913702; PMCID: PMC7052608 , Feb-2020