Fiscal Year:	FY 2015	**Task Last Updated:**	FY 11/17/2014
PI Name:	Natarajan, Mohan Ph.D.		
Project Title:	Targeting NO/IKK Signaling to Counteract Hemodynamic Flow-Dependent Endothelial Dysfunction and Vascular Damage after Space Radiation		
Division Name:	Human Research		
Program/Discipline:	NSBRI		
Program/Discipline--Element/Subdiscipline:	NSBRI--Cardiovascular Alterations Team		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **HHC**:Human Health Countermeasures		
Human Research Program Risks:	(1) **Cardiovascular**:Risk of Cardiovascular Adaptations Contributing to Adverse Mission Performance and Health Outcomes		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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City:	San Antonio	**State:**	TX
Zip Code:	78229-3901	**Congressional District:**	21
Comments:			
Project Type:	GROUND	**Solicitation / Funding Source:**	2011 Crew Health NNJ11ZSA002NA
Start Date:	11/01/2012	**End Date:**	10/31/2015
No. of Post Docs:	1	**No. of PhD Degrees:**	0
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NSBRI
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
Flight Assignment:	NOTE: Period of performance change to 11/1/2012-10/31/2015 per NSBRI; previous POP was 9/1/2012-8/31/2015 (Ed., 11/13/12)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Prihoda, Tom (The University of Texas Health Science Center at San Antonio) Mohan, Sumathy (The University of Texas Health Science Center at San Antonio) Blakely, Eleanor (Lawrence Berkeley National Laboratory)		
Grant/Contract No.:	NCC 9-58-CA02802		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	It is important to determine the effect of long-duration space flight on the heart and blood vessels and research ways to counteract those risks in order to subdue the onset/manifestation of any vascular abnormalities during the mission. In this study, we propose to test the hypothesis that space radiation at low doses may impair the interplay between three key proteins (eNOS, Hsp-90, and IKK-ß) in the vascular endothelial cells, the cell type that covers the inner lining of the blood vessels. This dysfunctional endothelium undergoes functional alterations. When unchecked, this may predispose the vascular bed to become a sustained pro-inflammatory milieu for the initiation of cardiovascular abnormalities. We proposed to address these above concerns by investigating how these molecular mediators are functionally interrelated and how they coordinately provide a niche for the development of cardiovascular abnormalities upon high LET radiation exposure. The objectives are: (i) to investigate the significance of high LET radiation on causing endothelial dysfunction and associated damages on vascular bed, impairment of cell migration/motility and inhibition of vascular healing processes. Three different HZE ion beams (16O, 28Si, and 56Fe) accelerated to the same velocity (600 MeV/amu) and having similar track structure dimensions, but different ionization densities will be compared; (ii) to study how high LET radiation concurrently exploits eNOS, Hsp-90, and IKK# signaling to cause endothelial dysfunction, while impairing the repair capacity of bone-marrow derived endothelial progenitor cells (EPCs); and (iii) to examine whether the findings, whilst allowing us to gain knowledge on the mechanism of cardiovascular alterations by high LET radiation exposure, would lead us to develop and quantitatively assess biological countermeasures for cardiovascular risks. This study emphasizes a multi-stage approach (in vitro, ex vivo, and in vivo) to understand the underlying mechanism of functional alteration of flow-adapted endothelial cells in response to space radiation. We have initially carried out parallel plate flow system at NSRL with a minimal facility in the absence of dedicated reach-in incubator and absence of CO2 atmosphere. At that time the medium were flushed with CO2 before assembled into the shear system at NSRL. In the current year we established a dedicated facility at NSRL that can be used to run four shear systems in parallel at-a-time under humidified CO2 atmosphere. The beam shape, beam uniformity, and other staging logistics at the beam path were optimized. Second, we progressed through the in vivo animal study to validate the results obtained from in vitro flow shear system. One wild type and two genetically modified (eNOS-/- and Tie2–eNOS) mice colonies with C57Bl/6 background were developed by standard breeding methods. The genotypes were confirmed with tail snip DNA analysis. When reached 18 months, the animals (n=8/group) were exposed to 0.8 Gy 56Fe (600 MeV/u) to (i) determine the radiation-altered regulation of vascular contractile and relaxation function and (ii) whether impaired eNOS/NO signaling after irradiation is responsible for those altered vasomotor function. The readouts comparing with the mock irradiated wild type, eNOS-/-, and Tie2–eNOS mice were carried out after 30 days post irradiation. Third, wild type C57Bl/6 mice were either mock irradiated or exposed to 0.8 Gy 56Fe (600 MeV/u) and used to determine the radiation-mediated impairment of endothelial progenitor cells in the bone marrow versus circulating blood. This approach was carried out to examine whether the back-up repair mechanism mediated through endothelial progenitor cells is also negated by the radiation and therefore unavailable to rescue the damaged cells on the vascular bed. Fourth, a pre-atherosclerotic model in wild type C57Bl/6 mice was developed by modulating the fluid dynamics of the artery. Carotid artery ligation was carried out on the right carotid artery near to bifurcation. The left carotid artery was used as control. The inflicted low shear region at the ligation site was anticipated to cause atherosclerotic lesion in three to four week. A total of 8 mice were irradiated (56Fe (600 MeV/u) and the sections of the carotid arteries were examined for lesions after 4 weeks post radiation. Fifth, in order to examine the IKK versus eNOS competition for Hsp-90 that was established through in vitro studies will also occur at the physiological condition, in vivo inhibition of IKK was carried out. Since the global blocking of IKK will result in an embryo with lethal phenotype, we developed IKK knockouts site-specifically at the vessel bed. Enriched endothelial cells from isolated arteries from IKK floxed mouse (IKKbf+/f+) and then transfected with cre-GFP were used to determine the influence of IKK in inhibiting eNOS binding to Hsp-90. Sixth, confirmatory experiments and additional data on polarization studies to biophysically prove the in vitro competitive binding of eNOS versus IKK were completed and submitted as a manuscript to Journal of Biological Chemistry (JBC). Finally, as a collaborative effort with another related cardiovascular project funded by NSBRI, the regulation of transcription factors NF-kB, STAT-3, and GATA were examined in cardiac tissue in mice exposed to 90 cGy of 1 GeV proton or 15 cGy of 1 GeV/u iron particle and sacrificed after 1, 3, 7, 14, and 28 days. From year two study we demonstrated the impaired regulation of nitric oxide signaling alters the physiological functioning of endothelium that leads to vascular abnormalities including activation of inflammatory responses, vasomotor function. Also, we confirmed the in vitro mechanistic studies and proved the proof of principal hypothesized in the original project (a full-length manuscript was submitted to JBC).
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	In the clinical set-up an increased risk of cardiovascular disease after radiotherapy is a major concern. Results from epidemiological studies clearly suggested that the cause of radiotherapy-induced vasculopathy leads to the induction or acceleration of atherosclerosis in conduit arteries located in the irradiated field. Second, therapeutic radiation while alleviating cancer burden can simultaneously be involved in the re-development of the disease at the treatment site. This may account for the tumor recurrence and raise the risk of metastasis at distant site. The approaches proposed in this current NSBRI project, once established will add significant advancement to the understanding of the mechanisms involved in two different diverse fields, i.e., cardiovascular disease and cancer after radiotherapy. Understanding the mechanism is important to develop targeted countermeasure approaches.

Task Progress:	This study addresses the concerns about whether space radiation affects endothelial cells which might initiate or accelerate coronary heart disease. In year 2 we progressed through the in vivo animal study. One wild type and two genetically modified (eNOS-/- and Tie2–eNOS) mice colonies with C57Bl/6 background were developed by standard breeding method. The genotypes were confirmed with tail snip DNA analysis. When reached 18 months, the animals (n=8/group) were exposed to 0.8 Gy 56Fe (600 MeV/u) to (i) determine the radiation-altered regulation of vascular contractile and relaxation function and (ii) whether dysfunctional endothelium with impaired eNOS/NO signaling after irradiation is responsible for those altered vasomotor function. The readouts comparing with the mock irradiated wild type, eNOS-/-, and Tie2–eNOS mice (n=8/group) were carried out after 30 days post irradiation. Second, wild type C57Bl/6 mice were used to determine the radiation (0.8 Gy 56Fe (600 MeV/u)-mediated impairment of endothelial progenitor cells in the bone marrow versus circulating blood. This approach was carried out to examine whether the back-up repair mechanism mediated through endothelial progenitor cells is also negated by the radiation and therefore unavailable to rescue the damaged cells on the vascular bed. Third, a pre-atherosclerotic model in wild type C57Bl/6 mice was developed by modulating the fluid dynamics of the artery. Carotid artery ligation was carried out on the right carotid artery near to bifurcation. The left carotid artery was used as control. The inflicted low shear region at the ligation site has been known to cause atherosclerotic lesion in three to four week in C57Bl/6 mice. A total of 8 mice were irradiated (56Fe (600 MeV/u) and compared to the mock irradiated controls (n=8). The sections of the carotid arteries were examined for lesions after 2-4 weeks post radiation. Fourth, in order to examine the IKK versus eNOS competition for Hsp-90 that was established through in vitro studies, will also occur at the physiological condition, in vivo inhibition of IKK was carried out. Since the global blocking of IKK will result in an embryo with lethal phenotype, we developed IKK knockouts site-specifically at the vessel bed. Isolated endothelial cells from IKK floxed mouse (IKKbf+/f+) transfected with cre-GFP were used to determine the influence of IKK in inhibiting eNOS binding to Hsp-90. Fifth, confirmatory experiments and additional data on polarization studies to biophysically prove the competitive binding of eNOS versus IKK were completed and submitted as a manuscript to Journal of Biological Chemistry (JBC). Finally, as a collaborative effort with another related cardiovascular project funded by NSBRI, the regulation of transcription factors NF-kB, STAT-3, and GATA were examined in cardiac tissue in mice exposed to 90 cGy of 1 GeV proton or 15 cGy of 1 GeV/u iron particle and sacrificed after 1, 3, 7, 14, and 28 days.
Bibliography Type:	Description: (Last Updated: 04/11/2021)
Awards	Manickam K. "Radiation Research Society Travel Award, May 2014." May-2014