Fiscal Year:	FY 2016	**Task Last Updated:**	FY 03/10/2016
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:	2	**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:	The development of subclinical vascular abnormalities, which have been known to occur during space missions, is largely due to functional alterations of endothelial cells (inner lining of the vessels). Endothelial dysfunction is regarded as a primary sub-clinical condition that could progress into cardiovascular diseases over the life of the astronauts. Our hypothesis is that space radiation at low doses may impair the interplay between three key proteins (eNOS, Hsp-90, and IKK-beta) and cause functional alterations of endothelial cells. This dysfunctional endothelium fails to regulate vascular healing processes and negates cell migration/motility. When unchecked, this may predispose the vascular bed to become a sustained pro-inflammatory milieu for the initiation of cardiovascular abnormalities. Radiation exposure can simultaneously also have an impact on endothelial progenitor cells (EPCs) and thereby attenuate EPC-dependent repair and re-endothelialization. The specific aims proposed in this study address the Human Research Program (HRP)-identified Integrated Research Plan (IRP) risks, gaps, and deliverables. In Aim 1, we proposed to study the significance of relevant doses of three qualitatively different high linear energy transfer (LET) radiation exposures on endothelial dysfunction that leads to vessel denudation, loss of migration and proliferation potential, inefficient damage-induced repair progression, and inept vascular wound healing. In Aim 2, we proposed to establish the intrinsic mechanism involved in radiation mediated endothelial dysfunction. The interplay between HSP-90, eNOS, and IKK-b will be examined. This will be validated with genetic and pharmacological blockers. As indicated in the HRP-Integrated Research Program road map, in Aim 3, we proposed to study the influence of countermeasure agents in limiting the radiation-related damage to endothelium. 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. 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. In this final year study on eNOS/NO signaling: We established space radiation (56Fe) at low doses (0.2 - 0.8 Gy) at 600 MeV energy could alter the delayed vascular function at molecular level (preferential binding of IKK-beta-Hsp-90 versus eNOS-HSP-90) cellular level (endothelial dysfunction), and tissue level (vascular relaxation/contractile function). Experiments revealed that eNOS activity is necessary but not sufficient to maintain the vasorelaxation function. Overexpression of vascular-specific expression of eNOS further worsens the relaxation function of the vessels in irradiated animals. We validated in vivo the potential countermeasure by duel approach of simultaneous upregulation of eNOS activity and at the same time negate the activity of IKK-ß with physiological eNOS inducer(s) and IKK-ß inhibitor(s), respectively. Currently there is no suitable animal model available to understand early biomarkers of radiation-induced cardiovascular complication. Since modulating the fluid dynamics of the artery has been shown to be associated with the accelerated atherosclerosis, we developed and optimized a partial ligation mouse model in wild type C57Bl/6 mice. Low or disturbed flow achieved in carotid artery by partial ligation will be used for the first time for radiation studies. Since the results were encouraging from the initial experiments with carotid artery ligation carried out last year, a second set of carotid artery ligation was carried out to determine the reproducibility. The results clearly showed infiltration of macrophages and intimal thickness that are responsible for atherosclerotic plaque formation. This novel approach, repurposed for radiation delayed effect, will help to understand whether or not the galactic cosmic radiation (GCR) mediates an accelerated atherosclerosis at low doses in normal individuals. Finally, a set of animals were exposed to 56Fe (600 MeV) at a total dose of 0.2 Gy for cardiotoxicity. In this experiment only the heart is targeted and rest of the body was shielded. Cardiac functional studies were performed in these mice after 3-6 months of post exposure period. Risk reduction & Gap closure: We have clearly identified the molecular mediators involved in space radiation-mediated vascular dysfunction. We have also proved that these molecular mediators are involved in vascular function in the arterial segments. These studies set a stage to move forward and validate the agents that target these selective mediators of the pathway as a potential countermeasure approach to alleviate the space radiation–induced endothelial-mediated vascular dysfunction. Recently we participated in a study as a co-author and submitted the findings to American Journal of Pathology - Heart and Circulatory Physiology. The manuscript was accepted for publication [Coleman MA, P Sasi SP, Onufrak J, Natarajan M, et al (2015) Am J Physiol Heart Circ Physiol 309: H1947–H1963, 2015] and was selected for podcast report. The corresponding author of this paper was invited to participate in AJP-Heart and Cir editorial Podcast. Part of this work was supported by the grant NSBRI - CA02802.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Increased longevity in cancer patients due to advancement in chemo and radiotherapy brought the awareness of possible therapy-induced vascular complications as one of the main latent side effects. Treatment-induced latent cardiovascular disease in surviving cancer patients is significantly limiting effective clinical management and quality of life. Being one of the primary cancer treatment modalities, the development of strategies that improve the efficacy of radiation therapy could benefit a significant number of cancer patients. Radio-therapeutic strategies can be significantly improved when the mechanism of therapy-induced cardiovascular effects are clearly understood and strategies developed to simultaneously intervene the involvement of adverse pathway mediators. The overall outcome from this study proposed to the National Space Biomedical Research Institute (NSBRI) will add a significant advancement towards treatment of cancer patients.
	Milestones 1. Established space radiation (56Fe) at low doses (0.2 - 0.8 Gy) at 600 MeV energy could alter the delayed vascular function at molecular level (preferential binding of IKK-beta-Hsp-90 versus eNOS-HSP-90) cellular level (endothelial dysfunction), and tissue level (vascular relaxation/contractile function). 2. Experiments revealed that eNOS activity is necessary but not sufficient to maintain the vasorelaxation function. Overexpression of vascular-specific expression of eNOS further worsens the relaxation function of the vessels in irradiated animals. We validated in vivo the potential countermeasure by duel approach of simultaneous upregulation of eNOS activity and at the same time negate the activity of IKK-ß with physiological eNOS inducer(s) and IKK-ß inhibitor(s), respectively. 3. Currently there is no suitable animal model available to understand early biomarkers of radiation-induced

Task Progress:	cardiovascular complication. Since modulating the fluid dynamics of the artery has been shown to be associated with the accelerated atherosclerosis, we developed and optimized a partial ligation mouse model in wild type C57Bl/6 mice. Low or disturbed flow achieved in carotid artery by partial ligation will be used for the first time for radiation studies. This novel approach repurposed for radiation delayed effect will help to understand whether or not the GCR mediates an accelerated atherosclerosis at low doses in normal individuals. Risk reduction & Gap closure We have clearly identified the molecular mediators involved in space radiation-mediated vascular dysfunction. We have also proved that these molecular mediators are involved in vascular function in the arterial segments. These studies set a stage to move forward and validate the agents that target these selective mediators of the pathway as a potential countermeasure approach to alleviate the space radiation–induced endothelial-mediated vascular dysfunction. Significant Media Coverage Recently we participated in a study as a co-author and submitted the findings to American Journal of Pathology - Heart and Circulatory Physiology. The manuscript was accepted for publication [Coleman MA, P Sasi SP, Onufrak J, Natarajan M, et al (2015) Am J Physiol Heart Circ Physiol 309: H1947–H1963, 2015] and was selected for podcast report. The corresponding author of this paper was invited to participate in AJP-Heart and Cir editorial Podcast. Part of this work was supported by the grant NSBRI - CA02802.
Bibliography Type:	Description: (Last Updated: 04/11/2021)
Articles in Peer-reviewed Journals	Coleman MA, Sasi SP, Onufrak J, Natarajan M, Manickam K, Schwab J, Muralidharan S, Peterson LE, Alekseyev YO, Yan X, Goukassian DA. "Low-dose radiation affects cardiac physiology: gene networks and molecular signaling in cardiomyocytes." Am J Physiol Heart Circ Physiol. 2015 Dec 1;309(11):H1947-63. Epub 2015 Sep 25. http://dx.doi.org/10.1152/ajpheart.00050.2015 ; PubMed PMID: 26408534; PubMed Central PMCID: PMC4698384 , Dec-2015
Articles in Peer-reviewed Journals	Krishnan M, Janardhanan P, Roman L, Reddick R, Natarajan M, van Haperen R, Habib S, de Crom R, Mohan S. "Enhancing eNOS activity with simultaneous inhibition of IKKß restores vascular function in Ins2(Akita+/-) type-1 diabetic mice." Lab Invest. 2015 Oct;95(10):1092-104. Epub 2015 Jul 27. http://dx.doi.org/10.1038/labinvest.2015.96 ; PubMed PMID: 26214584 , Oct-2015
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Articles in Peer-reviewed Journals	Natarajan M, Aravindan N, Sprague EA, Mohan S. "Hemodynamic flow-induced mechanotransduction signaling influences the radiation response of the vascular endothelium." Radiat Res. 2016 Aug;186(2):175-88. https://doi.org/10.1667/RR14410.1 ; PMID: 27387860 , Aug-2016