Fiscal Year:	FY 2010	**Task Last Updated:**	FY 07/26/2010
PI Name:	Berkowitz, Dan E. M.D.		
Project Title:	Ionizing Radiation and its Effects on Cardiovascular Function in the Context of Space Flight		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
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) **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		
PI Email:	dberkow1@jhmi.edu	**Fax:**	FY 410-955-0994
PI Organization Type:	UNIVERSITY	**Phone:**	410-614-1517
Organization Name:	The Johns Hopkins University		
PI Address 1:	Department of Anesthesiology & Critical Care Medicine		
PI Address 2:	600 North Wolfe Street		
PI Web Page:			
City:	Baltimore	**State:**	MD
Zip Code:	21287-8711	**Congressional District:**	7
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2004 Radiation Biology NNH04ZUU005N
Start Date:	07/01/2005	**End Date:**	12/30/2011
No. of Post Docs:	1	**No. of PhD Degrees:**	
No. of PhD Candidates:	1	**No. of Master' Degrees:**	
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	1
No. of Bachelor's Candidates:	3	**Monitoring Center:**	NASA JSC
Contact Monitor:	Cucinott1a, Francis	**Contact Phone:**	281-483-0968
Contact Email:	noaccess@nasa.gov		
Flight Program:			
Flight Assignment:	NOTE: End date changed per 11/11/2011 HRP Master Task List information (Ed., 2/2/2012) NOTE: New end date will be 6/30/2011, per C. Guidry/JSC (6/2010) NOTE: Received NCE to 12/31/2009 per J. Dardano/JSC (7/2009)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Nyhan, Daniel (Johns Hopkins) Shoukas, Artin (Johns Hopkins) Vazquez, Marcello (Brookhaven National Laboratory)		
Grant/Contract No.:	NNJ05HF03G		
Performance Goal No.:			
Performance Goal Text:			

Task Description:

An appropriate examination of the health risks associated with manned space flight necessitates an understanding of the molecular consequences of exposure to the radiations encountered in space. Human radio-epidemiologic data and animal studies indicate that irradiation of the heart can cause a spectrum of cardiovascular complications. The mechanisms suggested for these alterations are chronic inflammation induced by oxidative stress. It is well known that ionizing radiation (IR) produces biological damage by direct effect on DNA and indirectly by generation of reactive oxygen species (ROS) in the cellular milieu. The xanthine oxidoreductase (XOD) system is one of the major sources of free radicals in biologic systems. Since the XOD system is present primarily in the reduced XDH form in normal tissue, the production of free radicals is negligible. However, emerging data demonstrates that IR irreversibly converts the xanthine dehydrogenase (XDH) to xanthine oxidase (XO) leading to amplification and persistence of IR induced, ROS dependent cell damage. It is well known that ROS interferes with cellular signaling (nitrosylation and phosphorylation) and is pro-apoptotic (releases mitochondrial cytochrome-C and activates apoptotic pathways). One of the postulated mechanisms of radiation related tissue injury is endothelial cell damage. However little is known regarding other cellular and molecular targets in the pathophysiology of radiation-induced cardiovascular system dysfunction. Furthermore, little is known regarding the response of endothelial cells and cardiac myocytes to high LET (linear energy transfer) radiation. In this proposal we intend to use established in vivo and in vitro bioassays to characterize the radiation response to charged particle exposure. Furthermore, mechanistically we will focus on the interaction between ROS and nitric oxide (NO) pathways in the regulation of myocardial and vascular structure and function following oxidative stress (OS) induced by high LET radiation. Our group have demonstrated the important reciprocal interaction between NO and O2- (derived from XO) in the regulation of myocardial contractility and endothelial function. We will utilize our expertise to determine the effect of radiation on these important signaling pathways in the cardiovascular system. We hypothesize that charged particles will produce an acute oxidative stress event with cellular injury and possible death with early and late consequences that are dose, LET, and time-dependent. Endothelial and myocardial dysfunction represent integrated cumulative indicators of this cellular injury. We further hypothesize that radiation-induced endothelial and myocardial contractile dysfunction results from the specific imbalance in NO signaling induced by increased ROS production. In addition, we hypothesize that the XO, NOS (Nitric Oxide Synthase), arginase pathways play a critical role in the response to radiation-induced OS.
Therefore, our Specific Aims are:

Hypothesis 1: Charged particles (iron ions) will produce an acute oxidative stress event characterized by cellular and tissue injury expressed by endothelial and myocardial dysfunction.

Specific Aim 1: Time- and dose-responses for multiple indices of endothelial and myocardial function will be established in adult Wistar rats exposed to 600 MeV/n Fe (iron) beams at the NASA Space Radiation Laboratory, Brookhaven National Laboratory (BNL). Animals will studied non-invasively and tissues will be collected for histological, functional and molecular analyses using methods established in our laboratory at different time points. Indices of normal tissue function and homeostasis to be investigated include:

a) Endothelium: 1) vascular stiffness by Doppler effect using pulse wave velocity; 2) endothelial function in isolated vascular ring tissue and microvessels; 3) markers of apoptosis in vascular tissue.

b) Heart: 1) myocardial contractile function and contractile reserve in vivo; 2) contractility and contractile reserve in vitro in isolated cardiac myocytes; 3) markers of apoptosis in cardiac tissue (as above).

Hypothesis 2: Iron irradiation-induced endothelial and myocardial contractile dysfunction results from the specific imbalance in NO signaling induced by increased ROS production.

Specific Aim 2: To determine the whether low-fluences of iron ions alter the balance in NO signaling as a function of increased ROS production thereby impairing endothelial and myocardial function. Radiation doses will be selected based on results of Aim 1 and animals will be sacrificed for detailed analyses at various time points as in Aim 1. Vascular and heart tissues from adult Wistar rats exposed to 600 MeV/n Fe ions will be collected and we will measure:

1) NO bioavailability in vascular rings and NOx in plasma , 2) NOS activity using fluorescent dye in heart and blood vessels, 3) ROS levels using chemiluminescence and fluorescence bioassays, 4) Nitroso-tyrosine expression in vascular and cardiac tissue using Western blot analysis.

Hypothesis 3: XO, NOS, and arginase pathways play a critical role in the cardiovascular response to HZE particle radiation.

Specific Aim 3: Rats will be exposed to 600 MeV/n iron ions to determine the specific roles of XO, NOS and arginase in modulating cellular and tissue response to charge particle-induced oxidative stress. Radiation doses will be selected based on results of Aims 1-2 and animals will be sacrificed for detailed analyses at various time points as in Aim 1 for the following endpoints:

1) expression and activity of NOS, Arginase and XO at an RNA and protein level using quatitative PCR, Western blot and immunohistochemistry in heart and blood vessels; 2) Enzyme activity using specific inhibitors of each of the enzymes both alone and in combination with our in vitro vascular ring bioassay and isolated cardiac myocytes; 3) The effect of specific inhibitors on bioassays of ROS and NO (as in Aim 2). Hypothesis 4: Enzyme inhibitors and ROS scavengers will modulate early and late cardiovascular toxicity of low-fluences of iron ions.

Specific Aim 4: To determine if enzyme inhibitors and ROS scavengers can modulate the cardio-vascular effects of iron ions, Wistar rats and/or tissue preparations will be treated with enzyme inhibitors or ROS scavengers prior to and following 600 MeV/n Fe beam irradiation. We will use in vivo and in vitro bioassays of endothelial and myocardial function to test whether the XO inhibitor allopurinol, and the arginase inhibitors S-(2-boronoethyl)-L-cysteine (BEC), or difluoromethylornithine (DFMO) will attenuate radiation-induced cardiovascular effects.

While IR may have parallel effects on peripheral vasculature endothelium and cardiac contractile tissue, the interaction between the blood vessels and heart (ventricular-vascular coupling) has further profound effects on each of these systems. It is for this reason that an approach which incorporates both in vivo (integrated cardiovascular measures such as PWV and P-V loops), as well as isolated cellular and tissue measures of function is so important. Our methodologies will allow us to assess the contribution of each component (heart and vasculature) to the integrated system response to charged particle exposure.

Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Our research primarily studies space-related radiation effects. However, the majority of our iron-radiation studies are paired with similar studies investigating gamma-radiation biological effects. Gamma-radiation is a very prevalent source of radiation on earth, particularly in medical radiotherapy. Our research focuses on cardiovascular diseases and complications caused by radiation exposure. Many medical radiotherapies target the body core, where the heart and major veins and arteries are located. This is true in cardiac imaging techniques and treatment for cancers, such as Hodgkin's Disease. Thus, radiotherapy has potential to be very damaging to the cardiovascular system. Although our research has found high doses of gamma radiation to cause some vascular injuries, we are also interested in vascular protection. We are studying how large of a radiation dose a biological system can absorb before its defenses are overwhelmed. This knowledge would be very helpful in radiotherapy and occupational radiation exposure control. Also, we have identified a drug that can potentially protect against radiation injury. This can be very valuable in the cases of accidental radiation exposures, such as nuclear accidents. In conclusion, our research is very applicable to life on Earth.
Task Progress:	Our most current findings demonstrate that the aortic endothelium develops reduced migratory or proliferative capacity shortly after whole-body radiation exposure. The anti-angiogenic effect is maintained at later time points after radiation. Furthermore, a similar angiogenic impairment is produced with whole-body HZE Fe irradiation and by direct ex vivo irradiation of aorta. This may contribute to radiation-induced endothelial dysfunction or it may leave the vasculature vulnerable to future vascular injury. Our finding of significantly decreased cell outgrowth from aorta following radiation exposure can be explained by two mechanisms: 1) radiation-impaired endothelial proliferation or migration, or 2) radiation-induced endothelial cell death. Previous studies support both of these as potential mechanisms. In the coronary vasculature of Sprague-Dawley and Wistar rats, capillary density was diminished following 10, 15, 20, and 25Gy irradiation. This finding mimics pathological signs of heart disease and demonstrates a loss of angiogenic ability. On et al. observed reduced endothelial cell quantity in aortas of female Sprague-Dawley rats exposed to 10Gy gamma radiation after 1-week, through histological evaluation and immunoblotting for the endothelial-specific protein, von Willebrand factor. In contrast, a study of 45Gy irradiation of rabbit ear artery only reported changes in endothelial cell morphology 6 weeks after exposure, and no changes were detected following 10 and 20Gy doses. This same study showed a drastic decrease in endothelial nitric oxide synthase (eNOS) protein abundance 1-week post-irradiation. eNOS-produced nitric oxide is a critical signaling molecule promoting endothelial cell proliferation and health. In our current results, anti-angiogenic effects were observed at radiation doses much lower than those mentioned above. Furthermore, the data of Chapter 3 demonstrated that acute chemical treatments were able to fully restore aortic endothelial dependent relaxation after radiation, indicating that the endothelium is still present but in altered nitroso-redox state (decreased NO production and increased reactive oxygen species). This was confirmed through measurement of aortic NO and superoxide. Finally, our group (unpublished observation) and Oh et al. observed that radiation induces senescence in cultured endothelial cells, which is characterized primarily by arrested cell cycle and reduced proliferation. Considering these results, we hypothesize that the observed radiation-impaired cell outgrowth is due to reduced endothelial proliferation or migration as a consequence of impaired NO signaling. References: On YK, Kim HS, Kim SY, Chae IH, Oh BH, Lee MM, Park YB, Choi YS, Chung MH. Vitamin C prevents radiation-induced endothelium-dependent vasomotor dysfunction and de-endothelialization by inhibiting oxidative damage in the rat. Clin Exp Pharmacol Physiol. 2001;28:816-821. Oh CW, Bump EA, Kim JS, Janigro D, Mayberg MR. Induction of a senescence-like phenotype in bovine aortic endothelial cells by ionizing radiation. Radiat Res. 2001;156:232-240.
Bibliography Type:	Description: (Last Updated: 01/13/2014)
Articles in Peer-reviewed Journals	Soucy KG, Lim HK, Attarzadeh DO, Santhanam L, Kim JH, Bhunia AK, Sevinc B, Ryoo S, Vazquez ME, Nyhan D, Shoukas AA, Berkowitz DE. "Dietary inhibition of xanthine oxidase attenuates radiation-induced endothelial dysfunction in rat aorta." J Appl Physiol. 2010 May;108(5):1250-8. PMID: 20167676 , May-2010
Articles in Peer-reviewed Journals	Soucy KG, Attarzadeh DO, Ramachandran R, Soucy PA, Romer LH, Shoukas AA, Berkowitz DE. "Single exposure to radiation produces early anti-angiogenic effects in mouse aorta." Radiat Environ Biophys. 2010 Aug;49(3):397-404. Epub 2010 Apr 18. PMID: 20401726 , Aug-2010