| | | | |
|---|---|---|---|
| **Fiscal Year:** | FY 2012 | **Task Last Updated:** | FY 02/13/2012 |
| **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:** | 1 |
| **No. of PhD Candidates:** | 2 | **No. of Master' Degrees:** | 0 |
| **No. of Master's Candidates:** | 0 | **No. of Bachelor's Degrees:** | 1 |
| **No. of Bachelor's Candidates:** | 3 | **Monitoring Center:** | NASA JSC |
| **Contact Monitor:** | Simonsen, Lisa | **Contact Phone:** | |
| **Contact Email:** | lisa.c.simonsen@nasa.gov | | |
| **Flight Program:** | | | |
| **Flight Assignment:** | NOTE: End date changed per 11/11/2011 HRP Master Task List information (Ed., 2/2/2012)<br>NOTE: New end date will be 6/30/2011, per C. Guidry/JSC (6/2010)<br>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 )<br>Shoukas, Artin ( Johns Hopkins )<br>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.<br>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:** | Supplement to grant: Combined effect of Radiation and Hyperoxia on Cardiovascular Function<br>INTRODUCTION: Spaceflight missions entail up to 24 hours of extravehicular activity (EVA) per week. Astronauts are exposed to 100% oxygen during EVA activities in the space environment. The tissue response to hyperoxia in the space environment is influenced by exposure to radiation, mostly in the form of Galactic Cosmic Radiation. There are no obvious sequelae evident shortly after the EVA, however it is unknown if prolonged and repeated EVAs cause later tissue damage. Previously we have demonstrated that radiation induces an oxidative stress in vessels which leads to impaired endothelial function and vascular stiffness. We tested the hypothesis that hyperoxia represents an added or synergistic risk with radiation for vascular endothelial oxidative stress and dysfunction.<br><br>METHODS AND RESULTS: We developed an animal model incorporating 4 groups of mice: one group exposed to a hyperoxic environment, one exposed to radiation alone and one group exposed to hyperoxia in addition to radiation. A group without exposure to hyperoxia or radiation served as controls. Hyperoxia treatment was performed by exposing mice to >95% oxygen for 8 hours 3 times during one week in special chambers. Chambers have been designed and used by NASA to study hyperoxia / hypoxia in animal model. Radiation treatment was performed by exposing mice to 1 Gy gamma radiation at rate of 100 rad/minute. Mice were exposed for a period of 2 weeks. At the end of the 2 week period, terminal endpoints were determined 1) In vivo integrated cardiovascular function was measured using non-invasive Doppler to measure pulse wave velocity (PWV) 2) Ex-vivo endothelial function by measuring endothelial dependent vasodilation in aortic rings. Compared to unexposed animals PWV was increased both following exposure to hyperoxia and to radiation alone. Combined exposure to both hyperoxia and radiation resulted in additive effect and resulted in worse cardiovascular function as evidenced by maximum increase in PWV. In ex-vivo experiments of endothelial function we did not find any difference between control and exposed to radiation and hyperoxia groups.<br><br>CONCLUSION: Our data confirm that both hyperoxia and radiation adversely affect cardiovascular function and provides evidence that their negative effects are additive in nature.<br><br>FUTURE STUDIES: The current results in animal model provide sufficient evidence to justify future studies in astronauts. |
| **Bibliography Type:** | Description: (Last Updated: 01/13/2014) |
| **Articles in Peer-reviewed Journals** | Soucy KG, Lim HK, Kim JH, Oh Y, Attarzadeh DO, Sevinc B, Kuo MM, Shoukas AA, Vazquez ME, Berkowitz DE. "HZE 56Fe-ion irradiation induces endothelial dysfunction in rat aorta: role of xanthine oxidase." Radiat Res. 2011 Oct;176(4):474-85. Epub 2011 Jul 25. PubMed PMID: 21787183 , Oct-2011 |