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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:	Radiation exposure and the associated risks are important concerns in medical radiotherapy, occupational exposure, and manned space flight. Dose limits in particular fields have been established, but mostly in terms of excess cancer mortality. The risks of other fatal consequences and diseases still remain largely uncharacterized. Regardless, irradiation of the heart and vasculature has been implicated in the development of significant cardiovascular complications. While epidemiologic studies indicate a strong relationship between ionizing radiation and cardiovascular events, little is known about the pathobiology of this phenomenon. Consequently, potential therapies and countermeasures are sorely lacking. It is well known that radiation generates biological injury through DNA damage and production of reactive oxygen species (ROS). ROS is a critical signaling molecule at low levels, however, at high levels it can damage biomolecules, induce cellular death, and disrupt vital signaling pathways. In fact, oxidative stress is an accepted marker of poor vascular health observed in aging, hypertension, and other cardiovascular dysfunctions. More specifically, in the endothelial cell layer of the vasculature, ROS has been shown to scavenge the protective molecule, nitric oxide (NO). Through this common feature of radiation effect and cardiovascular disease, we have examined the molecular mechanisms of radiation-induced vascular injury and repair. We previously found radiation to damage vascular function in rats. We also determined the ROS producing enzyme, xanthine oxidase (XO), to contribute significantly to this damage. We continued to investigate if XO inhibition through a special diet could protect against radiation injury. In animals exposed to gamma radiation, this diet began 1 week before irradiation. In animals exposed to iron radiation, the diet began immediately after irradiation. In both cases, the XO inhibition diet provided significant protection for vascular function. After irradiation, we found that rat aorta could not relax tension as well and we determined that the aorta was stiffer than un-irradiated blood vessels. These are both symptoms of cardiovascular diseases, such as hypertension and aging. In the iron irradiated aorta, we did not observe any geometric changes in the vessels, indicating that mechanical properties of the aorta are responsible for the increased stiffness. Also, with gamma-radiation, we tested passive mechanical properties of aorta. Even after removing active muscle control, we found that the irradiated aorta was less compliant. All of these parameters were greatly improved with the dietary XO inhibition. We continued to investigate the reasons for these vascular problems. We determined that after radiation, rat aorta produced significantly less NO compared to un-irradiated aortas. Once again, the dietary inhibition of XO completely restored the NO production levels. In addition, after iron radiation these aorta also produced significantly more ROS. Through short-term XO inhibition, we could decrease this ROS production to amounts equal to un-irradiated aorta. We next look specifically at XO activity. After gamma radiation exposure, the XO activity was significantly elevated in rat aorta. There is also less damaging form of the XO enzyme, called xanthine dehydrogenase (XDH). The XDH activity was also increased, but to a lesser extent than XO activity. As a result, the XO-to-XDH ratio was greater in irradiated aortas, compared to un-irradiated aorta. As expected, dietary XO inhibition caused a decrease of XO and XDH activities, and the XO-to-XDH ratio. We have collected preliminary data showing an increase of both XO and XDH protein expression in response to radiation. Through continuing studies of XO activity and protein amount, we hope to determine the radiation-induced XO conversion mechanism. In conclusion, the inhibition of xanthine oxidase provides significant protection against radiation exposure. We also examined how radiation affected blood vessels' ability to produce new blood vessels, known as angiogenesis. To accomplish this we implemented an aortic angiogenesis assay in which aorta is embedded in a three-dimension biological substrate. After embedding the aortic sections, of both un-irradiated and irradiated rats, we would store the aorta in conditions to promote cell growth. After 4 days of incubation we would measure the cellular outgrowth from the aortic section. Before quantifying growth, we were able to determine that the cell outgrowth is endothelial dependent and that the cells sprouting from the aorta are mostly endothelial cells. We found that 1 day after single high dose of gamma radiation, the cell outgrowth was significantly reduces. This implies that blood vessels lose the ability to repair themselves or make new blood vessels after radiation injury. As a result, the vasculature is vulnerable to future complications. We will continue these studies and measure angiogenesis at lower radiation doses. Understanding radiation dose limits and thresholds is important for biological safety. These thresholds are most likely dependent on both damage pathways and endogenous protective mechanisms. Above, we described some potential damaging pathways in the cardiovascular system in response to radiation. However, we also investigated a potent antioxidant defense mechanism. When exposed to oxidative stress, a master transcription factor protein, Nrf2, moves from the cellular cytosol to the nucleus and binds with the antioxidant response element (ARE) on numerous antioxidant genes. As a result, numerous antioxidant genes are produced to provide defense against the oxidative stress that triggered the response. Using a cellular model of human aortic endothelial cells (HAEC) we found that gamma irradiation can cause the translocation of Nrf2 into the cellular nucleus. However, at our selected radiation dose range, we did not observe increased protein amounts for two of the downstream antioxidant proteins. We supported this finding with Taqman gene expression analysis. We did not see an increase of protein amount or gene expression of heme oxygenase or NAD(P)H:quinine oxidoreductase. We are interested if these genes can be induced at lower radiation doses. In summary, we've made significant progress in understanding the role of XO in radiation-induced damage. We also found that long term dietary inhibition delivers significant radiation protection. We've successfully implemented the established angiogenesis assay in our laboratory to assess endothelial damage and repair capability. In addition, we've acquired preliminary data indicating that the Nrf2 antioxidant defense system is either impaired by radiation or is not a

	viable defense system for radiation exposure, at least over our tested dose range. We plan to advance all these studies.
Bibliography Type:	Description: (Last Updated: 01/13/2014)
Abstracts for Journals and Proceedings	Soucy KG, Kim JH, Bugaj L, Ryoo S, Vandegaer KM, Nyhan D, Shoukas AA, Berkowitz DE. "Xanthine oxidase inhibition attenuates vascular endothelial dysfunction in irradiated rats." Presented at the NASA Human Research Program Investigators' Workshop, Houston, TX, February 4-6, 2008. NASA Human Research Program Investigators' Workshop, February 4-6, 2008. , Feb-2008
Abstracts for Journals and Proceedings	Soucy KG, Bhunia AK, Kim JH, Ryoo S, Vandegaer KM, Shoukas AA, Berkowitz DE. "Radiation impairs nitric oxide bioavailability and induces cellular damage through xanthine oxidase activation." Presented at the NASA Space Radiation Investigators' Workshop, Philadelphia, PA, June 30 - July 2, 2008. NASA Space Radiation Investigators' Workshop, Philadelphia, PA, June 30 - July 2, 2008. , Jul-2008
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Abstracts for Journals and Proceedings	Soucy KG, Bhunia AK, Chang F, Attarzadeh D, Romer LH, Shoukas AA, Berkowitz DE. "Radiation-induced endothelial dysfunction: Investigating the balance of injury and repair." Presented at NASA Human Research Program Investigators' Workshop, Houston, TX, February 2-4, 2009. NASA Human Research Program Investigators' Workshop, February 2009. , Feb-2009
Abstracts for Journals and Proceedings	Soucy KG, Bhunia AK, Attarzadeh D, Sevinc B, Chang F, Romer L, Shoukas AA, Berkowitz DE. "High-dose radiation produces anti-angiogenic effects potentially through impaired Nrf2-antioxidant defenses." Presented at Heavy Ions Symposium, Cologne, Germany, July 6-10, 2009. Heavy Ions Symposium, Cologne, Germany, July 6-10, 2009. , Jul-2009
Awards	Soucy KG, Bhunia AK, Attarzadeh D, Sevinc B, Chang F, Romer L, Shoukas AA, Berkowitz DE. "NASA Student Travel Award, July 2009." Jul-2009
Dissertations and Theses	Soucy KG. "Radiation Induces Vascular Dysfunction Through Xanthine Oxidase Activation." Thesis Proposal, Johns Hopkins University, May 2009. , May-2009