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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 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 will focus on the interaction between ROS and nitric oxide (NO) pathways in the regulation of both myocardial and vascular structure and function following 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 (linear energy transfer), 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) Nitrosotyrosine 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 Aim 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 SA2) .

Hypothesis 4: Enzyme inhibitors and ROS scavengers will modulate the 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 charge particle exposure.

Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	
Task Progress:	This report represents the report of the first year of the proposed studies. As such, our first animal experiments will take place this summer (06/23/06) for which we applied and were awarded beamline time at the Brookhaven National Laboratory. In the interim, we have conducted pilot experiments at the Johns Hopkins University Medical campus using conventional Gamma radiation facilities. Our findings are exciting and are consistent with our primary hypothesis. We hope to corroborate our findings in the pilot experiments. Below we describe the methods used in the conventional radiation studies. The endpoint measures are the same as will be used in the heavy ion radiation protocols. Male Sprague-Dawley rats were exposed to different doses of gamma-ray irradiation. Measurements of vascular stiffness, vasocontractility, vasorelaxation, and Xanthine Oxidase activity and expression were studied. Epidemiologic data and limited experimental data support the notion that radiation has significant effects on the cardiovascular system. These effects include vascular pathologies including accelerated atherosclerosis and hypertension, as well as primary myocardial dysfunction as a result of impaired myocytes contractility. Our current preliminary pilot study is one of the first studies in vivo and ex vivo to demonstrate that radiation induces changes in vascular stiffness (a well known independent predictor of cardiovascular events) as well as vascular endothelial dysfunction. Moreover, the impaired endothelial function could be reversed in vitro in the presence of the XO inhibitor oxypurinol. As highlighted in the background it is week established that XO is one of the primary sources of ROS in the cardiovascular system. In the blood vessels, an upregulation to XO contributes to endothelial dysfunction and vascular pathobiology in diabetes and aging. In the heart, and upregulation of XO contributes to the pathobiology of heart failure and the XO inhibitor markedly attenuates developed heart failure pathophysiology. Our pilot study supports the hypothesis that and activation/upregulation of XO may be an important/the important pathophysiologic consequence of radiation. The preliminary data demonstrated here is consistent with our original hypothesis. However, this data is observed with Low LET radiation. We await the results of the studies to be preformed at NSRL in the summer to confirm this hypothesis. If indeed upregulation of XO contributes to oxidative stress and endothelial dysfunction, XO may be a target for prevention and possible treatment of radiation-induced cardiovascular function. Presentations: 4th International Workshop on Space Radiation Research and 17th Annual NASA Space Radiation Health Investigator’s Workshop, Moscow and St. Petersburg, June 5-9, 2006. Abstract title: Single exposure gamma-irradiation amplifies xanthine oxidase activity and induces endothelial dysfunction in rat aorta. 2006 NASA Space Radiation Summer School Lecture: Cardiovascular Tissue Responses.
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