Monday, May 17, 2010

Poster Session I

Non-Cancer Effects

6:10 p.m. – 8:00 p.m.
Bayles and Willse Rooms
INTRODUCTION AND PURPOSE
The lens is a highly ordered tissue with unique optical properties and exquisite radiosensitivity. The goal of this project is to investigate the natural history of Rayleigh light scattering changes in pre-cataractous lenses of mice exposed to high-energy proton or iron particle radiation prevalent in space. This fully-validated, performance-tested, quasi-elastic light scattering (QLS) instrument was developed by Dr. Goldstein and colleagues at Boston University as a non-invasive molecular biodosimetry platform for detecting and monitoring exposure to biologically relevant space radiation. This innovative laser-based technology quantitatively assays the pre-cataractous molecular pathology of lenses in vivo. Particular emphasis has been placed on investigation of low particle radiation doses. We hypothesize that exposure to accelerated heavy ions will trigger a cascade of molecular and cellular events in the lens that can be detected in vivo by advanced light scattering technology instrumentation and confirmed by molecular and immunohistochemical techniques.

METHODS
To validate our QLS technology in an in vivo radiation model, we utilized an available cohort of C57Bl6 male mice (25 males/group) that were irradiated with 10 or 100 cGy of 1.0 GeV/amu protons (LET~0.22 keV/μm) or 1 GeV/amu iron ions (LET~155 keV/μm) at the NASA Space Radiation, Brookhaven National Laboratory (BNL). Control mice (n=10 males) were sham irradiated. The mice were followed and examined monthly by QLS. Baseline QLS was conducted on all mice prior to irradiation. For each examination, the left eye was dilated (1% tropicamide) and the lens assessed by QLS without anesthesia. Each examination consisted of 15 autocorrelation acquisitions with infrared slit-lamp imaging of the sampled lens region. Autocorrelation and scattering intensity analyses were conducted on data averaged over each 15-acquisition test session and compared to conventional slit lamp biomicroscopy conducted on the same mice. Analytical polystyrene bead (0.2, 0.1, 0.05 μm) analytical standards were used for longitudinal QLS instrument calibration and intra-assay precision analysis. At the end of the experiment, mice were sacrificed and the lens tissues were collected for postmortem analysis.

RESULTS AND CONCLUSIONS
We demonstrated intra-assay precision of the QLS platform and successfully acquired longitudinal autocorrelation functions and Raleigh scattering intensity values from non-anesthetized mice in irradiated and sham cohorts. QLS and slit-lamp examinations were compared. Non-invasive QLS instrumentation for in vivo lens assessment has been successfully optimized to study the longitudinal effects of space radiation exposure in mice. We anticipate that results from this project will contribute to our understanding of the early processes of protein aggregation and molecular pathology associated with radiation damage in the lens.

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Age, Gender, and Hormones as Determinants of Cataractogenesis Induced by Low and High LET Radiation

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Recent studies of astronauts and cosmonauts suggest that exposure to even relatively low doses of space radiation may result in a reduced latent period for, and an increased incidence of cataractogenesis. Astronauts exposed to low or high linear energy transfer (LET) radiation during interplanetary or prolonged lunar missions may therefore be at an increased risk of developing cataracts. However, the determinants of cataractogenesis are not clearly understood. Previously, we demonstrated that exposure of the eyes of male Sprague-Dawley rats to low LET radiation (10 Gy of 60Co γ-rays) resulted in an increased incidence of cataracts when compared to female rats. This gender difference was not due to differences in estrogen, since male rats treated with the major secreted estrogen, 17-β-estradiol (E2) showed an identical increase compared to untreated males. We have recently finished accumulating data that allow us to compare the incidence and rate of progression of cataracts induced by high LET radiation in male and female rats. Rats received a single dose of 1 Gy of 600 MeV 56Fe ions to the right orbit, and were examined by slit lamp biomicroscopy to measure anterior and posterior subcapsular lens opacification every 2-4 weeks. While the incidence and rate of progression of radiation-induced cataracts was significantly increased in the animals in which estrogen was available from endogenous or exogenous sources, we found that male rats with E2 capsules implanted one week prior to irradiation had significantly higher rates of progression compared to male rats with empty capsules implanted, but not compared to the intact female rats. These results contrast with data obtained after low LET irradiation. Interestingly, we also identified a major difference in the age response for radiation cataractogenesis when progression and incidence of opacification was compared after irradiation of eyes with low vs. high LET radiation. One-year old female rats were either left ovary-intact, ovariectomized, or ovariectomized and implanted with a silastic capsule containing 20 mg of E2 one week prior to exposure of the right eye to 2 Gy of 600 MeV 56Fe ions. Eyes of 56-day old ovary-intact rats were also irradiated. The rate of progression of cataractogenesis was significantly greater and the latent period reduced in the irradiated eyes of 1 year old rats compared to young (56 day old) rats. Furthermore, similar to what we observed for young rats, older rats that received continuous exogenous estrogen treatment (17-β-estradiol) commencing 1 week prior to irradiation and continuing throughout the period of observation showed an increased incidence of cataracts and faster progression of opacification compared to intact rats with endogenous estrogen or ovariectomized rats. However, the more rapid onset and progression of cataracts in 1 year old rats compared to 56 day old rats was independent of estrogen status, suggesting that estrogen cannot account for the age-dependent differences in cataractogenesis induced by high LET radiation. These data are in direct contrast to the results obtained after irradiation with low LET radiation, as we found that young rats showed a greater incidence of cataracts after exposure to 10 Gy of 60Co γ-rays compared to older rats. We speculate that the different types of damage caused by high and low LET irradiation may be influenced differentially by steroid sex hormones, and that while E2 is modulatory, hormones other than estradiol may account for age- and gender-specific differences in radiation cataractogenesis.
HZE AND SKELETAL RECOVERY FROM SIMULATED WEIGHTLESSNESS

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Prolonged spaceflight causes bone loss in astronauts which leads to compensatory remodeling after return to earth. In space, radiation fields also may have detrimental consequences due to the damaging effects of charged particles on both progenitors and mature cell populations within actively remodeling tissues such as bone. The effects of heavy particle radiation exposure in combination with weightlessness on bone cells and tissue are not well understood. Exposure of mice to a relatively high dose of HZE ($^{56}$Fe, 1GeV/n, 2Gy, total body irradiation) stimulates resorption of metabolically active cancellous bone by osteoclasts, and causes tissue loss over 3 to 30 days. Furthermore, we showed HZE at a lower dose (50cGy) inhibits growth and differentiation of bone-forming osteoblasts from marrow progenitors (osteoblastogenesis), but only if mice also were also unloaded at the time of irradiation. Given these combined effects, we hypothesize HZE impairs skeletal remodeling as the mice age and prevents compensatory changes following simulated weightlessness. Four mo. old, male C57Bl/6J mice that were normally loaded (NL) were irradiated with $^{56}$Fe (1GeV/amu) then tissues harvested 6 mo. later; age-matched groups were hindlimb unloaded (HU) to produce musculoskeletal disuse for 11 days, irradiated, released 3 days later to ambulate normally, then tissues harvested at the same age as NL mice. In NL mice, irradiation with 50 cGy did not alter fractional bone volume (an indicator of bone density) measured by 3D microcomputed tomography when compared to sham-irradiated, age-matched controls. In contrast, if mice were hindlimb unloaded, irradiation with the same dose (50cGy) caused a 29-32% reduction in fractional bone volume compared to NL and HU sham-irradiated controls (P<0.001, 1-factor ANOVA, Fisher PLSD). Detailed microarchitectural analyses revealed that transient unloading alone caused a persistent loss of trabeculae (the struts that comprise cancellous tissue) compared to NL controls. Irradiation of HU mice with 50cGy reduced fractional bone volume apparently by impairing thickening of the residual trabeculae during the 6-month period of re-ambulation. Ex vivo marrow cultures revealed 50cGy and 200cGy profoundly inhibited osteoblastogenesis. In contrast to adverse effects on cancellous tissue, no changes were evident in the cortical shell. We conclude that 50 cGy $^{56}$Fe prevented cancellous recovery from disuse in the long term, which is likely to be caused by lasting damage to osteoprogenitors and stem cells in the marrow. This leads us to speculate that there is a potential for HZE radiation in space to prevent compensatory skeletal remodeling after return to earth; by extension, repair and remodeling associated with age-related diseases in other tissues also may be vulnerable.

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STS-118 Spaceflight Effects on Expression of Liver Genes Associated with Metabolism and Tissue Remodeling

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INTRODUCTION
As human presence expands beyond low Earth orbit, there is critical need for a better understanding of health consequences inherent to the spaceflight environment. Studies of crewmembers on shuttle missions and the MIR space station have found depressed plasma protein synthesis (Aviakosm Ekolog Med 34:12-16, 2000; Aviat Space Environ Med 77:745-48, 2006) and elevated levels of interleukin-6 in urine (Am J Physiol 266:E448-52, 1994), indicating that the liver was affected by mission-related stressors. The liver is an essential organ responsible for numerous biological activities including detoxification of potential carcinogens, production of glucose and hormones such as insulin-like growth factor 1, and breakdown of insulin. Our overall hypothesis was that significant changes would be readily measurable in expression of genes related to metabolic disorders and pathological conditions involving tissue remodeling shortly after return from a flight on the space shuttle.

MATERIALS AND METHODS
C57BL/6NTac female mice (n = 24; Taconic Farms, Inc., Germantown, NY) were shipped to the NASA Space Life Sciences Laboratory (SLSL) at Kennedy Space Center at ~7 wk of age. Animal enclosure modules (AEM) with food bars and water were used to house flight mice (FLT, n = 12) and ground controls (AEM, n = 12). Mice were adapted to the food bars, water system, and raised mesh floor for 1 wk. The FLT mice flew onboard the Space Shuttle Endeavour (STS-118; CBTM-2 payload experiment) for 13 days. Muscle strength testing and nuclear magnetic resonance (NMR) measurements were performed by Amgen investigators prior to euthanasia in 100% CO2 within 3-5 h after landing. Liver tissue was quick-frozen in liquid nitrogen at SLSL and shipped overnight to Loma Linda University (LLU). RNA extraction was done at LLU using standard procedures; quantitative RT-PCR was performed at SABiosciences service core (Frederick, MD). Two arrays, 84 genes each, were used: Insulin Signaling Pathway (PAMM-030) and Tumor Metastasis (PAMM-028). The data presented here represent 3 mice/group; fold-change in gene expression for FLT mice compared to AEM controls was evaluated using Student’s t test.

RESULTS
The Insulin Signaling Pathway array focused on insulin-responsive genes and genes involved in metabolism of carbohydrates, lipids and proteins. Analysis showed that 21/84 of these genes were significantly up-regulated and 4/84 genes were down-regulated in livers from the FLT mice compared to AEM ground controls (p<0.05). Those up-regulated by >2-fold were Frap1, Gdp1, Grb2, Grb10, Jun, Pklr, Pkcc, Pkcz, Ptpn1, Serpine1, Slc2a1 and Tg; >2-fold down-regulated genes were Acaca and Tg. The genes in the Tumor Metastasis array encoded cell-to-cell adhesion molecules, transmembrane receptors, matrix metalloproteinases, cell cycle regulators and selected cytokines/chemokines. The data showed that 7/84 of these genes were significantly up-regulated by flight and 20/84 were down-regulated (p<0.05). A >2-fold change was noted in Ewsr1, Fli4, Kiss1 and Myc (up-regulated) and in Cd44, Il1b, Il8rb, Mycl1, Mmp13, P2ry5, Pten and Set (down-regulated).

CONCLUSIONS
The results demonstrate that the gene expression profile was significantly altered in livers from mice shortly after return from a 13-day flight in space. Insulin and many of the proteins encoded by the tissue remodeling/signaling genes mediate a wide spectrum of biological responses that can influence risk for diabetes, obesity, hypertension, and cardiovascular disease. It should also be noted that two well-known proto-oncogenes (Jun and Myc) were among the most highly up-regulated. However, much more study is obviously needed to determine if any of the noted aberrations persist long-term and translate into adverse health consequences.

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Mechanisms of Ocular Cataracts

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To date, increased incidence and earlier onset of cataract are the only long-term degenerative effects observed in some astronauts exposed to space radiation. This finding might be explained by individual genetic susceptibility. Individuals that are haplo-insufficient for one or more genes involved in determining radiosensitivity may be more susceptible to the cataractogenic effects of ionizing radiation than wild-types. In addition, considerable uncertainty still remains surrounding the relationship between radiation dose and quality and the risk of lens opacification.

This NASA funded project has been examining the influence of genetic heterogeneity on radiation cataract development after ocular exposure to either low-LET X-irradiation or high-LET heavy ions. Previous findings have demonstrated that mice haplo-insufficient for Atm develop high-LET radiation induced cataracts earlier and at a higher grade than wild type animals. In addition, recent work with AtmRad9 double heterozygotes has demonstrated that the cataractogenic effect of combined heterozygosity is greater than for each gene alone. More recently, we have begun examining the effect of genetic heterogeneity for singly or doubly haploinsufficient Atm and Brca1 mice on radiation cataract after exposure to either 500 mGy X-ray or 50 and 250 mGy ⁵⁶Fe. In addition to determining the radiosensitivity of the single or double heterozygotes, we have also compared the RBE for x-ray cataractogenesis to that of heavy ions for each genotype. Lastly, we have generated a number of new mutant mice combinations, including those doubly haploinsufficient for Atm and either p53, p21 or PTEN and triply haploinsufficient for Atm, p53 and p21. All these heterozygous genotypes are viable and have been maintained for more than six months. Recent studies suggest expression of these genes, which have important roles in recognizing and repairing radiation induced DNA damage, inducing cell cycle arrest and initiating apoptosis, are interrelated.

These studies provide an opportunity to study the influence and effects of genetic heterogeneity in an organized tissue, the lens, in a genetically defined mouse model that has great relevance and similarity to human response to radiation exposure and determination of appropriate human exposure guidelines. These findings are likely to shed light on the genetic control and cellular mechanisms of both heavy ion and proton induced cataractogenesis. More importantly, using the lens as a model system, these findings may have important implications for radiosensitive subsets of the human population, including the astronaut core, and aid in determining future national space radiation risk policies.

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Radiation exposure from a number of sources is associated with an increased risk for atherosclerotic disease. For example, even relatively young patients, who would otherwise be at very low risk, have an increased incidence of stroke after having undergone radiation therapy for head and neck cancer. Similarly, major causes of death for atomic bomb survivors include myocardial infarction and stroke. For workers exposed at Chernobyl, the most significant adverse effect was cardiovascular disease. Even radiation technologists working before 1950 (when shielding was less rigorous) had an increased incidence of myocardial infarction.

Although these risks are well established by epidemiologic data, the risk of deep-space travel is difficult to predict. Unlike most terrestrial radiation, which consists primarily of gamma- and X-ray photons, cosmic radiation has a large component of ions. The effect of this cosmic radiation on tissues can be very different, and sometimes much more damaging, than the forms of radiation with which we are more familiar. Moreover, iron ions (\(^{56}\)Fe) present particular challenges due to their high energy and propensity to interact with shielding to generate secondary particles.

Therefore, to determine whether cosmic radiation is a risk factor for atherosclerosis, we irradiated mice with 2 to 5 Gy of 600 MeV iron ions at Brookhaven National Laboratory (BNL). For these experiments, we chose to use the apoE \(-/-\) mouse model of atherosclerosis because they are an established model of radiation-induced atherosclerosis, and, unlike wild type mice they develop atherosclerosis as they age, without special diets or other interventions, as do humans. Radiation was targeted to the upper aorta and the carotid arteries only. In this way, direct effects of radiation on blood vessels could be assessed, without complications arising from systemic radiation effects on the bone marrow or most other organs. Mice were then dissected 13 weeks post-irradiation to determine whether exposure had accelerated development of atherosclerosis. We found the degree of atherosclerotic disease to be greater in the aortic arch, aortic root, and carotid arteries of irradiated mice as compared to both unirradiated portions of vessels and to un-irradiated control mice.

Since some exposure is likely to be unavoidable, it was important to attempt to determine the molecular mechanism of these effects in order to form a basis for developing possible pharmacological or dietary countermeasures. Therefore, a second arm of the study consisted of irradiation of tissue cultures of the endothelial lining of these vessels. To closely model the response of astronaut vessels, primary human aortic endothelial cells were used. All adhesion assays were performed in a flowchamber assay designed to mimic conditions of shear stress inside blood vessels.

We found that both iron ions and X-rays increased the adhesiveness of aortic vascular endothelium at the same doses as those that accelerated atherosclerosis. Antibody blocking experiments confirmed that this adhesion was mediated by the leukocyte integrins typically associated with atherosclerosis-related adhesion. With the adhesion
molecules identified, current efforts are aimed at determining how their function is modulated by radiation to increase adhesiveness. A better understanding of this mechanism will lead to possible therapeutic targets, with the potential to develop countermeasures not only against cosmic radiation effects, but to mitigate the long-term effects of terrestrial exposure as well.

TRAIL IS A SENSITIZER FOR PROSTATE CANCER CELLS TO RADIATION THERAPY

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Background: Prostate cancer (PCa) is the most commonly diagnosed and second leading cause of cancer related death in men in the United States. The advanced PCa and bone metastasis is refractory to conventional therapy, including radiation therapy (i.e. usually 30 Gy in 10 fractions). Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) induces apoptosis in tumorigenic cells without adversely affecting normal cells. We tested if TRAIL can sensitize PCa cells to radiation therapy. Methods: We developed a prostate restrictive replication competent recombinant adenovirus armed with TRAIL (AdE4PSESTRAIL). We analyzed the prostate specific replication, the expression levels of membrane and soluble TRAIL protein, and the in vitro and in vivo antitumor effects (i.e. apoptosis and survival) of TRAIL and radiation. Results: AdE4PSESTRAIL is a prostate-restricted replication competent adenovirus in PSA/PSMA-positive cell lines. PSA/PSMA-positive PCa cells expressed both membrane and soluble TRAIL protein after virus infection. The TRAIL protein made the significant bystander apoptotic effect in the neighboring PSA/PSMA-negative PCa cells. When AdE4PSESTRAIL was combined with radiation at a series of doses, we observed AdE4PSESTRAIL works synergistically with radiation for enhanced in vitro PCa cell killing (p<0.01). Importantly, the antitumor efficacy of radiation was significantly augmented when combined with AdE4PSESTRAIL in a PSA/PSMA-positive prostate cancer xenograft model. Conclusions: TRAIL can sensitize PCa cells to radiation therapy. The combination of TRAIL and external beam radiation treatment can be potentially used as a novel and powerful radiation sensitizing approach in future clinical setting.