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Research Activities at the Loma Linda University and Proton Treatment Facility - An Overview

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Abstract

The Loma Linda University (LLU) Radiobiology Program coordinates basic research and proton beam service activities for the university and extramural communities. The current focus of the program is on the biological and physical properties of protons and the operation of radiobiology facilities for NASA-sponsored projects.

The current accelerator, supporting facilities and operations are described along with a brief review of extramural research projects supported by the program. These include space craft electronic parts and shielding testing as well as tumorigenesis and animal behavior experiments. An overview of research projects currently underway at LLU is also described. These include: 1) acute responses of the C57Bl/6 mouse immune system, 2) modulation of gene expression in the nematode *C. elegans* and rat thyroid cells, 3) quantitation of dose tolerance in rat CNS microvasculature, 4) behavioral screening of whole body proton and iron ion-irradiated C57Bl/6 mice, and 5) investigation of the role of cell integration into epithelial structures on responses to radiation.

KEYWORDS: Proton, Radiobiology, Immune System, Behavior.

1. Program Direction

NASA and Loma Linda University are participating in a Cooperative Research Agreement to characterize the effects of proton radiation on biological systems in order to provide a scientific basis for risk assessment of astronauts unavoidably exposed to charged particles in space and to improve charged particle-based medical treatment modalities. Under this agreement Loma Linda scientists: 1) conduct collaborative research into the genetic, cellular and systemic effects of protons, 2) provide assistance to visiting investigators who require proton radiation for their studies, and 3) operate the facilities needed to conduct proton radiation research.

2. Facilities

A dedicated research beam line is available at the Loma Linda University synchrotron facility for exposing biological samples, shielding materials, dosimetric instruments and electronic parts to protons of single energies between 40 and 250 MeV or to mechanically-modulated beams of complex spectra. Multi-angle exposures can also be performed on patient treatment gantries. Exposure regimens are coordinated with the Brookhaven National Laboratory AGS biology collaboration to assure high fidelity comparisons of protons with accelerated high Z ions. Staging laboratory and logistics equipment facilitate movement between proton or gamma ray exposure areas and laboratories for preparation and analysis of samples. Core laboratories provide access to state-of-the-art analytical equipment for cell and molecular biology and complement an AALAC-accredited animal facility. Upgrades to accelerator systems will provide capabilities for long duration low dose rate experiments using large fields to better simulate realistic space radiation exposures of animals, tissues and cells. These will include improved scattering and low fluence dosimetry, limited beam scanning, control system upgrades and environmental support equipment for cells and animals

3. Research Activities

Collaborative research and pilot studies are addressing the effects of protons on genome integrity and gene expression, cell organization into functional tissue units, immune system function, tissue changes in rodent central nervous systems and behavioral toxicity of radiation.

The immune system of whole body proton-irradiated C57Bl/6 mice has been characterized for acute and late alterations in specific lymphoid cell populations, spontaneous and induced blastogenesis, T-cell mediated cytolysis, hematology profile, spleen and thymus histological changes and profiles of cytokine production. Quantitation of micronuclei provides a measure of fixed genetic damage in immune cells. The effects of dose rate and spacecraft-like shielding on these parameters have been measured. Using hematopoietic stem cells of CBA and C57Bl/6 mice (which vary in their predisposition to late expression of cytogenetic damage) the stability of the genome following proton irradiation is being investigated in collaboration with Dr. Munira Kadhim (MRC, Harwell, UK). Flow cytometric analysis of mature and developing blood cells complements analysis of chromosome aberrations to understand the mechanism(s) underlying the late expression of the radiation-induced damage.

Stereological techniques have been applied to

quantitation of microvasculature cell kinetics following irradiation of rat brain regions and retinas with highly collimated proton beams. Endothelial and pericyte population changes have been described out to two years post irradiation using clinically-relevant dose fractionation schedules. As a measure of CNS functional changes, a series of behavior pilot studies have been conducted with C57Bl mice to evaluate radiation toxicity of protons and accelerated iron ions. Open field behavior, acoustic startle and rotor rod assessments have been used to evaluate spontaneous activity, startle reflex and motor coordination. Dose vs. response relations were described for the first time. Water maze experiments are in progress for evaluation of spatial memory in collaboration with Dr. Robert Pearlstein (Duke University) and collaborators.

The importance of cell integration into functional tissue units in determining sensitivity and specificity of responses to irradiation is being examined using the rat FRTL5 thyroid cell line which organizes into functional follicle homologues in culture. Control of tissue structure by gap junctions and integration with extracellular matrix are being evaluated along with the functions of protein kinase C isoforms and stress responsive signal transduction pathways. Laser scanning cytometry techniques have been developed to enhance quantitative data collection for cell cycle redistribution, apoptosis and surface receptor abundance's.

Gene expression patterns following irradiation of FRTL5 cells and *C.elegans* nematodes have been mapped using differential display reverse transcriptase PCR techniques. Accelerated iron ions, protons and cobalt gamma rays show unique patterns of gene expression at 3 to 6 hours post irradiation. Over 400 amplified gene products showing significant changes in abundance post irradiation have been preliminarily identified. Sequencing of 75 amplicons from nematodes and FRTL5 cells followed by sequence database searches reveals an abundance of genes from chaperone and cellular oxidative stress management families. Confirming hybridization experiments are in progress. Together these studies represent a com-

Table I – Highlights of Accelerated Proton Radiation Investigations Conducted at Loma Linda University.

Test Subject	Measurement	Organization
Electro-optical coupler for Cassini space craft imaging photometer	Failure mode in integrated circuits	Jet Propulsion Laboratory Pasadena, CA
International Space Station medical ultrasound system	Failure mode in integrated circuits and software	NASA Johnson Space Center, Houston, TX
U.S. and Russian extravehicular activity space suits with Loma Linda tissue phantom	Shielding of suits and components exposed to protons and electrons. Spectrometric, thermoluminescence and ion chamber measurements with tissue phantom.	NASA Johnson Space Center, Houston, TX, University of San Francisco, San Francisco, CA Lawrence Berkeley Laboratory, Berkeley, CA
Human lymphocytes	Cytogenetics following high and low dose rate exposures and shielding	NASA Johnson Space Center, Houston, TX
Rats	Mammary tumorigenesis	Johns Hopkins University, Baltimore, MD
Rats	Behavioral conditioning	University of Maryland, Baltimore, MD
Mice	Hematopoietic stem cell chromosome aberrations	Medical Research Council, Harwell, UK
Microdosimetry instruments	Short range target fragments	Colorado State University Fort Collins, CO
Cultured lymphoblastoid cells	Mutations	Lawrence Berkeley Laboratory Berkeley, CA
Cultured cells	Protein kinase expression	University of California, Riverside, CA

The table summarizes a partial list of the investigations conducted at the Loma Linda University Proton Treatment Facility and supported by the Radiobiology Program. Indicated are the types of sample, the endpoints measured and the organization performing the investigation.

prehensive approach to understanding the unique effects of proton irradiation at the molecular, cell, tissue and systems levels.

4. Support to Outside Investigators

A variety of proton radiation investigations by scientists from other universities and national laboratories have been supported by the Radiobiology Program. These range from quality assurance measurements on satellite electronics to shielding by spacesuits and tumorigenesis in rats. A sample of some of these activities is summarized in Table I.

5. Summary

Working with NASA and scientists supported by the Space Radiation Health Program, Loma Linda's Radiobiology Program has developed a robust user facility that has provided service to investigators conducting physics, material science and biology investigations using accelerated protons. Radiobiological investigations conducted at Loma Linda in collaboration with others have probed the unique effects of proton radiation on gene expression, genotoxicity, cell survival and tissue organization, immune system function and rodent behavior. Capabilities are being extended to low dose rates and large fields capable of high fidelity simulations of space radiation environments.

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