Non-Invasive Early Detection & Molecular Analysis of Low X-ray Dose Effects in the Lens

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Purpose: The lens is a highly-ordered tissue with unique optical properties and exquisite radiosensitivity. The focus of this project is to evaluate radiation cataract dose response and mechanisms associated with low-linear energy transfer (LET) X-rays. We aim to investigate the natural history of Rayleigh light scattering changes in pre-cataractous lenses of mice exposed to radiations using a fully-validated, performance-tested quasi-elastic light scattering (QLS) instrument developed by Dr. Goldstein and colleagues at Boston University. This innovative laser-based technology quantitatively assays pre-cataractous molecular pathology in the lenses of living mice exposed to X-ray or sham radiation. Particular emphasis will be placed on investigation of low dose radiation. This proposal dovetails with a complementary NASA-funded project led by Dr. Eleanor Blakely on using the same technology to investigate the effects of proton-, high LET heavy ion irradiation on early molecular markers for cataractogenesis in mice. We hypothesize that exposure to ionizing radiation 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 biochemical and immunohistochemical techniques. These effects may be mediated in part by reactive oxygen species generated in the local tissue microenvironment.

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: 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.

Conclusions: Non-invasive QLS instrumentation for *in vivo* lens assessment has been successfully deployed to study the longitudinal effects of space radiation exposure in mice. Plans to establish calibration standards by irradiating proteins in solution and exposing animal to low doses of X-rays are in progress. 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.