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

DE- SC0002213, PI: Lee Goldstein, MD, PhD, Boston University School of Medicine, Boston, MA

PROJECT OVERVIEW & SIGNIFICANCE

This is the Final Progress Report for DOE-funded research project DE- SC0002213 titled "*Non-Invasive Early Detection and Molecular Analysis of Low X-ray Dose Effects in the Lens*". The project focuses on the effects of low-linear energy transfer (LET) radiation on the ocular lens. The lens is an exquisitely radiosensitive tissue with a highly-ordered molecular structure that is amenable to non-invasive optical study from the periphery. These merits point to the lens as an ideal target for laser-based molecular biodosimetry (MBD). Following exposure to different types of ionizing radiations, the lens demonstrates molecular changes (e.g., oxidation, racemization, crosslinkage, truncation, aggregation, etc.) that impact the structure and function of the long-lived proteins in the cytosol of lens fiber cells. The vast majority of proteins in the lens comprise the highly-ordered crystallins. These highly conserved lens proteins are amongst the most concentrated and stable in the body. Once synthesized, the crystallins are retained in the fiber cell cytoplasm for life. Taken together, these properties point to the lens as an ideal system for quantitative *in vivo* MBD assessment using quasi-elastic light scattering (QLS) analysis.

In this project, we deploy a purpose-designed non-invasive infrared laser QLS instrument as a quantitative tool for longitudinal assessment of pre-cataractous molecular changes in the lenses of living mice exposed to low-dose low-LET radiation compared to non-irradiated sham controls. We hypothesize that radiation exposure will induce dose-dependent changes in the molecular structure of matrix proteins in the lens. Mechanistic assays to ascertain radiation-induced molecular changes in the lens focus on protein aggregation and gene/protein expression patterns. We anticipate that this study will contribute to our understanding of early molecular changes associated with radiation-induced tissue pathology. This study also affords potential for translational development of molecular biodosimetry instrumentation to assess human exposure to mixed radiation fields.

SCIENTIFIC JUSTIFICATION

Expression of radiogenic cataract phenotypes are dependent on radiation type, dose and dose rate. Recent epidemiological and animal studies suggest a low- or no-dose exposure limit for radiogenic cataracts. *Despite over one hundred years of research and hundreds of reports documenting the effect of ionizing radiation on the lens, the molecular and cellular mechanisms underpinning development of radiogenic changes in the lens remain largely unknown.* Even less is known about exposure mixed radiation fields (such as might be expected in the vicinity of an accidental or intentional nuclear incident) and low-dose exposure dependency. This project addresses these interconnected scientific questions that are of direct relevance and fundamental importance to the field of low-dose radiation biology.

PROJECT PROGRESS & TECHNICAL DEVELOPMENTS

This is the Final Progress Report.

Specific Aim 1: *To investigate the effect of X-ray irradiation on lens protein aggregation in vivo.*

For our *in vivo* mouse irradiation experiment, C57/Bl6 male mice were X-ray irradiated at 3-months old using a new X-ray irradiator (XRAD 320, Precision X-Ray, Inc., North Branford, CT) at Boston University School of Medicine at exposure doses (i.e., 0, 10, 20, 100, 400 cGY) and have added a high-dose cohort (11 Gy) as a positive control. The addition of the high-dose group

is necessary to maximize data interpretability across all the study cohorts, and additionally, to provide a well-established biological control for instrument signal calibration. At this high dose (11 Gy), we expect fully penetrant expression of classical radiogenic cataractogenesis by 3 months following radiation exposure in the C57/B6 mouse strain (Pendergrass et al., Molecular Vision, 2010; endpoint analysis in this study was conducted by *ex vivo* lens phenotyping). Since our study follows an *in vivo* longitudinal study design, inclusion of this high-dose cohort: (i) ensures *in vivo* phenotype expression by 3 months post irradiation, and (ii) provides a means to evaluate the very earliest time points in which pre-cataractous radiogenic lens changes might be detectable using our extremely sensitive QLS technology. If we did not include this positive control, we would be unable to ascertain whether negative results were truly negative or the result of instrumental and/or systematic error. Note that our exposure paradigm does not include body shielding except for the 11Gy dose which is otherwise lethal.

STUDY NOTE #1: *An important corollary of our central hypothesis is that exposure to ionizing radiation induces dose-dependent radiolytic damage to the long-lived matrix proteins within the cytoplasm of the lens fiber cells.* If this molecular damage hypothesis is correct, we should be able to detect direct evidence of radiation-induced protein damage within a short time period (days to weeks) following exposure. This expected early change in QLS response represents a quantitative early molecular phenotype (biomarker) reflecting radiolytic damage to the cytosolic structural proteins within the lens fibers cells. By contrast, classical radiogenic cataracts (PSC) represent a late cellular phenotype resulting from radiation-induced chromosomal damage, alterations in gene expression profiles, and metaplastic changes involving the lens epithelium. Validating this pathogenic distinction is a central focus of this project.

STUDY NOTE #2: *The ongoing nuclear exposure threat in Japan provides a timely and important practical justification for inclusion of a high-dose control cohort.*

When the mice reached 21 months old, the mice were sacrificed and the eyes were processed as follows:

The Left eye was dissected and the lens was imaged by ex-vivo slit lamp. The lens was then snap-frozen. These lenses are being analyzed for gene expression changes using the Extracellular Matrix and Adhesion Molecules RT^2 profiler PCR array (SABiosciences, Qiagen) by Drs Blakely and Chang at Lawrence Berkeley Natl Lab under their subcontract to this grant.

The right eye was fixed in 10% neutral buffered formalin and will be analyzed for reactive oxygen and nitrogen species (ROS/RNS) activity. Due to our delay in irradiating mice (as we awaited a new X-ray instrument to be setup at Boston University), we have not yet completed all our analyses of tissues but these are currently underway and we expect a publication from this. In addition, retinas and brains from these mice were collected but further funds not budgeted in this DOE grant would be necessary to perform analyses on these tissues studying in particular the effects of low-dose X-ray on retinal and brain pathology.

Regarding our QLS results, we have had some confounding results as our control sham group developed age-related lens opacities as early as 5-6 months of age. This has confounded our ability to analyze and compare our QLS results to the irradiated mice. Looking further into this problem and working with the animal vendor (Charles River Laboratories), we have discovered that the C57Bl/6 mouse strain has some endogenous susceptibility to eye problem such as micro-ophthalmia but in the past we excluded these animals. However, over the years as the animals have become more inbred, it appears that this ocular phenotype has become more common and pronounced as a consequence of genetic drift. In our Alzheimer's disease study, we were able to use QLS to differentiate between wild-type mice and mice that express the Swedish mutant

human Alzheimer's disease gene that also express the Alzheimer's disease-linked supranuclear cataract in transgenic mice with a B6SJL background that did not have eye problems in the corresponding wildtype strain. Consequently, in our Alzheimer's disease study, control animal lenses remained clear of opacities for up to ~15-16 months of age and were then only affected in very few animals. Therefore in future studies, it might be advisable to use a different mouse strain background such as the B6SJL. Regardless of this issue, the post-mortem analyses of the eye tissues for gene and protein changes due to radiation effects is uncompromised.

Specific Aim 2: *To investigate the effect of X-ray irradiation on lens protein aggregation ex vivo.*

We have performed in vitro experiments using X-ray at Boston University/Lawrence Berkeley National Laboratory as part of an ongoing collaboration with Drs. Eleanor Blakely and Polly Chang. In this study, we used human lens protein extract from a 62-yr old male and split the sample into X-ray irradiated (30Gy) and non-irradiated. We followed the sample over time taking QLS measurement for 110 days (Figure 1).

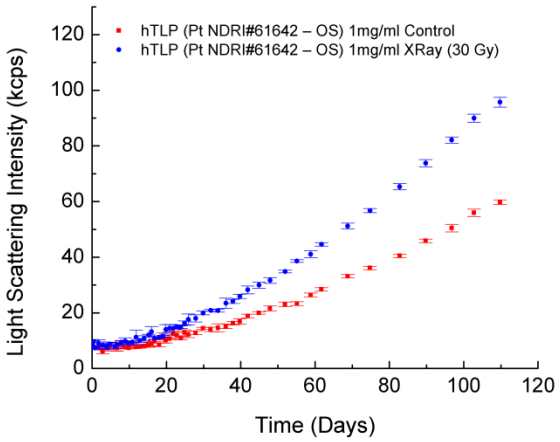

Figure 1

In the first 20 days, there was no significant difference between the X-ray irradiated sample and the control. However after 20 days, the difference in QLS intensity become significant with the irradiated sample showing increase intensity compared to the control.

Furthermore, we have developed another analytical technique that allows us to analyze the QLS signals to give us information of size distributions of particles in our samples. In the above described experiment, we were able to detect at day 110 a shift in increased particle size in the X-ray sample compared to the control (Figure 2).

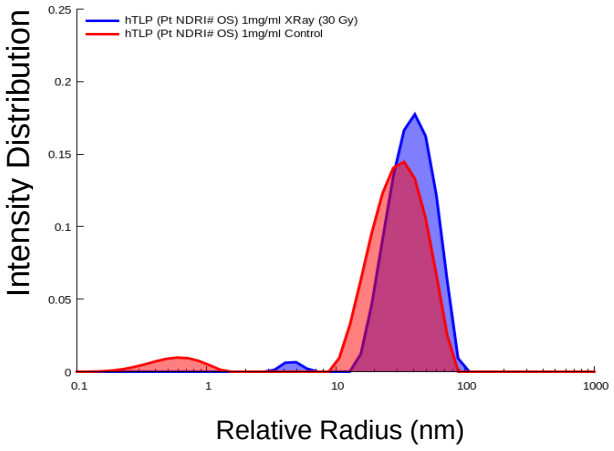

Figure 2

We also discovered that the increase in light scattering from human lens protein extract is radiation dose-dependent (Figure 3) and concentration-dependent (Figure 4 and 5).

Our results demonstrate that changes are occurring in the lens following X-ray irradiation and these changes are detectable by QLS in the lens but it takes time to observe significant changes (up to 20 days in the experimental setup) and the X-ray dose is relatively high (30 Gy). We speculate that lowering the dose would mean that detecting these changes may require even longer time periods to see a significant change in QLS signal and size distribution changes.

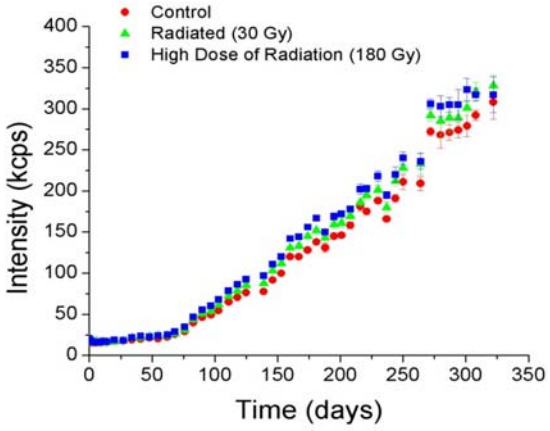

Figure 3

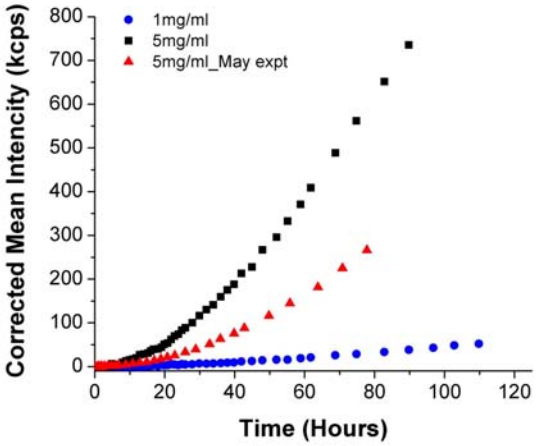

Figure 4

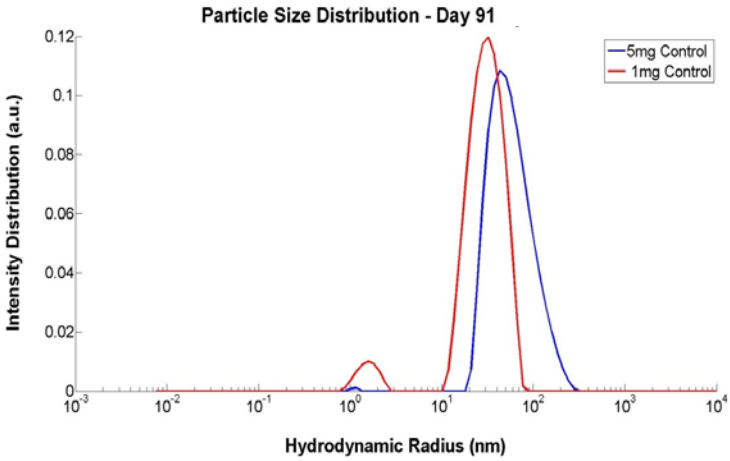

Figure 5

PROJECT PLANS & FUTURE ACCOMPLISHMENTS

As mentioned above, retinae and brains from the mice described above and exposed to 0, 10, 20, 100, 400 cGY and 11 Gy were collected and banked. Additional funds would be necessary to perform analyses on these tissues. We are particularly interested in studying the effects of radiation on the brain as it correlates with our current NASA-funded grant (NNX11AR05G, PI: Goldstein): *Effects of Space Radiation on Hippocampal-Dependent Learning and Neuropathology in Wild-Type and Alzheimer's Disease Transgenic Mice.*

ANTICIPATED RESULTS & SIGNIFICANCE

We anticipate that the data generated during the course of this study will allow, for the first time, a clinicopathological analysis of pre-cataractous molecular changes in the lens and mapping of the natural history and molecular pathology of this radiosensitive tissue following exposure to low-dose X-ray irradiation. Importantly, the proposed study complements ongoing NASA-funded research by establishing additional databases for comparative and correlation analyses of molecular changes in the lens in response to high-energy, low-LET protons, or high-energy, high-LET heavy ion irradiation.

Please briefly (16000 chars or less) summarize your most recent results to date: *

Specific Aim 1: *To investigate the effect of X-ray irradiation on lens protein aggregation in vivo.*

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(ROS/RNS) activity. Due to our delay in irradiating mice (as we awaited installation of a new X-ray instrument at Boston University), we have not yet completed all tissue analyses but these are currently underway and we expect a publication. In addition, retinas and brains from these mice were collected but further funds not budgeted in this DOE grant would be necessary to perform analyses on these tissues studying in particular the effects of low-dose X-ray on retinal and brain pathology.

Specific Aim 2: *To investigate the effect of X-ray irradiation on lens protein aggregation ex vivo.* We have performed in vitro experiments using X-ray at Boston University-Lawrence Berkeley National Laboratory using human lens protein extract. Our results demonstrate that changes are occurring in the lens and are detectable by QLS in the lens but it takes time to observe significant changes (up to 20 days in the experimental setup) and the X-ray dose is relatively high (30 Gy). We speculate that lowering the dose would mean it would take even longer than 20 days to see a significant change in QLS signal and size distribution changes.

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In this project, we deployed a purpose-designed non-invasive infrared laser QLS instrument as a quantitative tool for longitudinal assessment of pre-cataractous molecular changes in the lenses of living mice exposed to low-dose low-LET radiation compared to non-irradiated sham controls. We hypothesize that radiation exposure will induce dose-dependent changes in the molecular structure of matrix proteins in the lens. A novel aspect of the present study focuses on animal age at irradiation, an issue of fundamental importance to the field. Mechanistic assays to ascertain radiation-induced molecular changes in the lens will focus on protein aggregation and gene/protein expression patterns. We anticipate that this study will contribute to our understanding of early molecular changes associated with radiation-induced tissue pathology. This study also affords potential for translational development of molecular biodosimetry instrumentation to assess human exposure to mixed radiation fields.

SCIENTIFIC JUSTIFICATION

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PROJECT PROGRESS & TECHNICAL DEVELOPMENTS

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Furthermore, we have developed a further analytical technique that allows us to analyze further the QLS signals to give us information of size distributions of particles in our samples. In the above described experiment, we were able to detect at day 110 a shift in increased size of particles in the X-ray sample compared to the control.

Our results demonstrate that changes are occurring in the lens and are detectable by QLS in the lens but it takes time to observe significant changes (up to 20 days in the experimental setup) and the X-ray dose is relatively high (30 Gy). We speculate that lowering the dose would mean it would take even longer than 20 days to see a significant change in QLS signal and size distribution changes. However, as we have also learned, using human lens samples vary from one sample to another even if using the same age group. Therefore as in this experiment, it is really important to use the same sample from the same patient when studying radiation vs non-irradiated controls.

PROJECT PLANS & FUTURE ACCOMPLISHMENTS

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ANTICIPATED RESULTS & SIGNIFICANCE

We anticipate that the data generated during the course of this study will facilitate development and implementation of noninvasive ophthalmic technology for clinicopathological analysis of pre-cataractous molecular changes in the lens and mapping of the natural history and molecular pathology of this radiosensitive tissue following exposure to low-dose X-ray irradiation. Importantly, the proposed study complements ongoing NASA-funded research by establishing additional databases for comparative and correlation analyses of molecular changes in the lens in response to high-energy, low-LET protons, or high-energy, high-LET heavy ion irradiation.