Effects of dietary ω-3 Polyunsaturated Fatty Acids (n-3 PUFA) in light sensitivity of retinas of mice models to prevent retinal damage

Aaron Rice
(931) 494-4429, arice20@uthsc.edu
UTHSC Doctor of Medicine Candidate, 2025

Faculty Advisor:
Nawajes Mandal, Ph.D.
Professor
Departments of Ophthalmology, Anatomy and Neurobiology
The University of Tennessee Health Science Center
7th Floor, 930 Madison Avenue, Memphis, TN 38103
(901) 448-7740, nmandal@uthsc.edu

Research conducted at 930 Madison Ave, Memphis, TN 38103; suite 718

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Abstract:

Light-induced retinal degeneration (LIRD) causes photoreceptor cell death in albino mice after exposure to high intensity light for a set period of time (6-24 hours). This causes retinal photoreceptor cell death through apoptosis. From several previous studies, Dr. Mandal's lab concluded that de novo biosynthesis of ceramide mediates photoreceptor cell death in a LIRD model. Previous studies in Dr. Mandal's lab has shown that mouse models with higher endogenous ω-3 Polyunsaturated Fatty Acids (n-3 PUFA) generates less ceramide upon neuronal injury and prevent neurodegeneration (Mol Neurobio 2021). There are currently not many available effective therapies for retinal degeneration in humans that have inherited diseases leading to blindness. We speculate that higher endogenous n-3 PUFA will prevent retinal degeneration in mouse model of light induced retinal degeneration.

This project aimed to see a significant difference between LD-Control vs LD-PUFA. Results showed that there was a significant difference between NLD-Control vs LD-Control and NLD-PUFA vs LD-PUFA. Similar levels of degeneration of retinal photoreceptors occurred in n-3 PUFA and control. n-3 PUFA has shown inconclusive results for decreasing photoreceptor cell death. Further testing should be done to confirm the significance of the effect of n-3 PUFA in a mouse-model.

1 Introduction:

Light-induced retinal degeneration (LIRD) causes photoreceptor cell death in albino mice after exposure to high intensity light for a set period of time (6-24 hours). This causes retinal photoreceptor cell death through apoptosis. Retinal cell death through apoptosis caused by LIRD is similar to retinal degeneration in humans.[1] The close similarity between the retinal degeneration of the albino mice and humans have sparked extensive studies of the mechanism behind the retinal photoreceptor death. From several previous studies, Dr. Mandal's lab concluded that de novo biosynthesis of ceramide mediates photoreceptor cell death in a LIRD model.[2] Ceramide is a sphingolipid that can be produced through many different pathways and act as a second messenger in retinal cell death. The main pathway of ceramide production in retinal cells are orchestrated by serine palmitoyl transferase. Other pathways use sphingomyelinase to convert sphingomyelin to ceramide, and another pathway hydrolyzes complex sphingolipids such as cerebrosides and gangliosides, until it eventually forms ceramide. The last pathway to consider is the salvage pathway which utilizes recycled sphingosine to produce ceramide.

Previous studies in Dr. Mandal's lab has shown that mouse models with higher endogenous ω-3 Polyunsaturated Fatty Acids (n-3 PUFA) generates less ceramide upon neuronal injury and prevent neurodegeneration (Mol Neurobio 2021). Previous studies have shown that members of the n-3 PUFA family, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have potential therapeutic effects since they play a role in controlling inflammatory pathways.[6, 14-16] We speculate that higher endogenous n-3 PUFA will prevent retinal degeneration in mouse model of light induced retinal degeneration.

Hypothesis:

Enrichment of ω-3 Polyunsaturated Fatty Acids (n-3 PUFA) in the retina of a mouse model will protect the retina from light induced retinal degeneration (LIRD), preventing acute damage to the retina.

Significance:

There are currently not many available effective therapies for retinal degeneration in humans that have inherited diseases leading to blindness. Analyzing potential compounds that could be used in the development for a therapeutic strategy could potentially slow down or prevent retinal degeneration. We identified that sphingolipid ceramide is a potential second messenger for retinal degeneration. There are available drugs such as fumonisin B1, myriocin, and FTY720. However, these drugs have systemic effects which are unfavorable.[2,3] n-3 PUFA can be used as a potential therapeutic, as well as a natural supplement as an augmentative therapy with potentially fewer side effects.

2 Methods

Animals:

Albino Blabc mice will be involved in this project. The experiments performed will follow the protocol approved by UTHSC's Institutional Animal Care and Use Committee.

Diet of Animals:

Groups of WT C57BL/6 mice will be fed an ω-3 Polyunsaturated Fatty Acids (n-3 PUFA)-enriched diet from birth. These diets will be prepared from Dyets Incorporation by supplementing AIN-93G purified rodent diet with n-3 PUFA, 20% lipids supplied by EPA and DHA (2:1 EPA:DHA ratio), which is commercially available in liquid form (Dr. Sears' OmegaRx 2 Fish Oil Liquid from Zone Labs Inc., Peabody, MA 01960). Groups of WT mice fed with the control diet (AIN-93G) will serve as controls for the n-3 PUFA group.

Light Induced Retinal Degeneration (LIRD) and Data collection:

Adult (2 months +) albino mice on the ω-3 Polyunsaturated Fatty Acids (n-3 PUFA)-enriched diet and the control animals will be born and raised in a dim light room (5-10 lux) and exposed to 1,500 lux white light for 6 hours to induce LIRD. Unexposed mice from both the groups will serve as controls. Mice will be transferred to their native dim room and retinal function will be tested using electroretinography after 7 days.

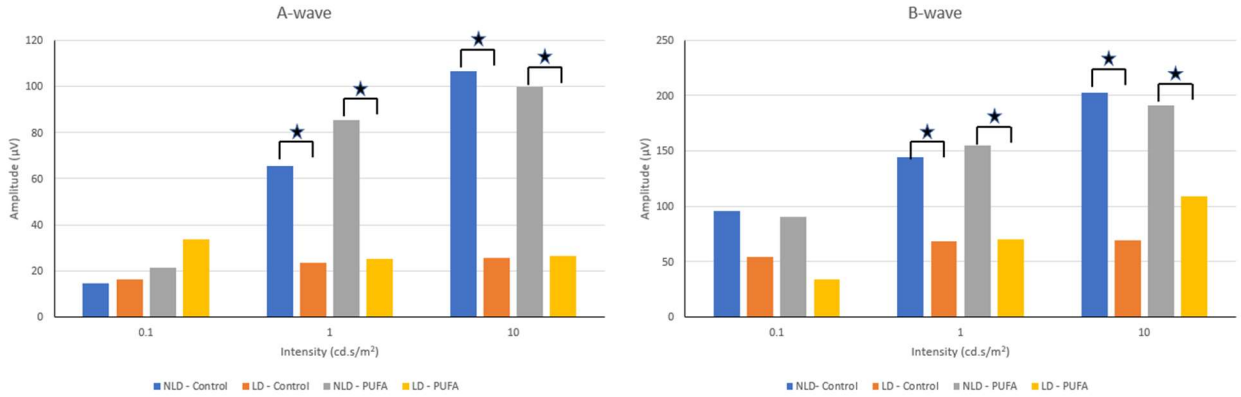
Electroretinography (ERG):

Electroretinography allows us to analyze the function of the rod and cone photoreceptors after albino mice are exposed to light as described in previously published studies. Mice were dark adapted overnight and were prepared for ERG under dim red light. They were anesthetized with ketamine (100mg/kg) and xylazine (5mg/kg) body eight via intraperitoneal (IP) injection. For dilation of the pupil, a drop of 1% (w/v) Atropine and 1% (w/v) Tropicamide (Akorn Inc., Lake Forest, IL) along with a drop of 0.5% (w/v) Proparacaine HCl (Alkon Lab, Fort Worth, TX) as local anesthetics were applied on the cornea. Throughout the whole procedure, a heating pad at 37°C was used to keep the mice warm and maintain their body temperature. For ERG measurement, a designated gold electrode was placed on the cornea, a reference electrode was positioned on the head, a ground electrode was placed at the tail, and the mouse was placed in a Ganzfeld illuminating sphere. For scotopic ERG, three strobe flash stimuli were presented at flash intensities of 0.1, 1, and 10 cd.s/m^2. The amplitude of the a-wave was measured from the pre-stimulus baseline to the a-wave trough. The amplitude of the b-wave was measured from the trough of the a-wave to the peak of the b-wave.

Histology:

After recording data with electroretinography, the histology of the albino mice's retina can be analyzed by following a procedure done in Dr. Mandal's lab. The eyes can be harvested and cut along the vertical meridian through the optic nerve into five micron-thick sections. The cut sections can be analyzed microscopically after staining the sections with hematoxylin and eosin. Biochemical assays of the retinas from independent groups of animals harvested at 0 hours and 6 hours after exposure to light from the treated and control groups will be conducted by lipidomics, qRT-PCR, and Western blotting.

Results:



4 groups of Balb/c albino mice with n=3 in each separated into Non-light damage (NLD-Control), Light damage (LD-Control), Non-light damage-PUFA (NLD-PUFA), Light damage-PUFA (LD-PUFA). Mice were exposed at 3 different intensity levels: 0.1, 1, and 10 cd.s/m2. Statistical significance was found to be between NLD-Control vs LD-Control and NLD-PUFA vs LD-PUFA.

Discussion:

Results showed that there was a significant difference between NLD-Control vs LD-Control and NLD-PUFA vs LD-PUFA. This project aimed to see a significant difference between LD-Control vs LD-PUFA. However, there was not a statistical significance even though prior

research has strongly suggested that n-3 PUFA should have a systemic effect on the inflammatory pathway which should include the retina. A possible reason for not seeing any statistical significance could have been that the project only had n=3 for the results. Unfortunately, biological experiments can run into many barriers, postponing results. Our other experiments to increase the number of n ran into an error that led to growing another group of Balb/c albino mice for future experiments 2-3 months into the future. Once these experiments are done, it is possible that there may be statistical significance between the LD-control vs LD-PUFA groups.

Conclusion:

Similar levels of degeneration of retinal photoreceptors occurred in n-3 PUFA and control. n-3 PUFA has shown inconclusive results for decreasing photoreceptor cell death. Further testing should be done to confirm the significance of the affect of n-3 PUFA in a mouse-model.

Citations:

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