The integral membrane protein adiponectin receptor 1 (AdipoR1) is necessary to retain docosahexaenoic acid (DHA) and to sustain photoreceptor cell (PRC) integrity. The molecular principles for DHA uptake/retention in retinal pigment epithelial (RPE) cells and PRC are not understood. Here we demonstrate that the seven trans-membrane domain AdipoR1 protein, which displays an extracellular C terminal and thus is not a G protein nor does it signal as a G protein, regulates the DHA lipidome, and its ablation in mice triggers PRC degeneration.

**Methods:** Two independent lines of AdipoR1 KO mice were developed by gene trapping and homologous recombination that lead to PRC degeneration. The role of AdipoR1 in PRC biology was explored by ERG, optical coherence tomography (OCT), histology, biochemistry, and LC-MS/MS-based lipidomics.

**Results:** In situ hybridization shows AdipoR1 in PRC/RPE, whereas no specific signal was in mice lacking AdipoR1. This KO showed:

- a) progressive PRC degeneration (3–33 weeks of age by OCT and histology), attenuated ERGs, prolonged dark recovery and impaired retinol visual cycle; b) flecked retina resembling human fundus albipunctatis with intact vasculature (12–16 week-old); c) anti-F4/80-positive cells (activated macrophages) beneath the RPE, UV autofluorescence in RPE and macrophages, and undigested outer segment debris in RPE (by EM); d) TUNEL-positive cells in outer nuclear layer; e) specific reduction of retinal DHA, since arachidonic acid (esterified and free) and systemic DHA were unchanged; f) decreased DHA uptake in eye cups/RPE incubated with deuterium labeled-DHA; g) overexpression or silencing of AdipoR1 in human RPE cells leading to enhanced or decreased DHA uptake, respectively; and h) absence of PRC-specific very long chain polysaturated fatty acids (VLC-PUFAs) along with unchanged ELOVL4 abundance.

**Conclusions:** AdipoR1 is a novel molecular switch, independent of its cognate ligand adiponectin, that selectively controls the DHA lipidome in RPE cells and PRC. Moreover, this switch modulates DHA retention and conservation, and is required for PRC-specific elongation to VLC-PUFAs. Since the PRC DHA lipidome comprises endogenous cell survival responses, mimicking them to counteract early stages of retinal degenerative diseases will lead to a therapeutic paradigm shift.

**Commercial Relationships:**
- Nicolas G. Bazan, None; Dennis S. Rice, None; William C. Gordon, None; Jorgelina M. Calandria, None

**Support:** NIH Grant EY005121; NIH Grant GM103340; Research to Prevent Blindness

**Program Number:** 4359

**Presentation Time:** 11:00 AM–11:15 AM

**Purpose:** The molecular principles for DHA uptake/retention in retinal pigment epithelial (RPE) cells and PRC are not understood. Here we demonstrate that the seven trans-membrane domain AdipoR1 protein, which displays an extracellular C terminal and thus is not a G protein nor does it signal as a G protein, regulates the DHA lipidome, and its ablation in mice triggers PRC degeneration.

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- Nicolas G. Bazan, None; Dennis S. Rice, None; William C. Gordon, None; Jorgelina M. Calandria, None

**Support:** NIH Grant EY005121; NIH Grant GM103340; Research to Prevent Blindness
Program Number: 4361
Presentation Time: 11:30 AM–11:45 AM
A diet supplemented with grapes preserves photoreceptor function in mouse models of retinal degeneration

Maria Esperanza E. Rodriguez Escalante, Tinthu Lee, Ashley Davis, Amit K. Patel, Abigail Hackam. Bascom Palmer Eye Institute, University of Miami, Miami, FL.

Purpose: Several studies have suggested neuroprotective benefits from specific diet supplementation in patients with age-related macular degeneration. The effect of diet on photoreceptor survival in mouse models of retinal degeneration has not been described. In this study, we tested whether a diet supplemented with grapes regulates photoreceptor function and survival in two mouse models of retinal degeneration.

Methods: The rd10 genetic model of retinitis pigmentosa was analyzed at post-natal days (P)18, P24 and P32. In the acute oxidative stress model, photoreceptor injury was induced by subretinal injection of 1 mM paraquat (PQ) in C57Bl/6 mice, and the mice were analyzed at 2 weeks post injury. Mice were fed the grape-supplemented diet in the form of chemically defined freeze-dried grape powder (FDGP), sugar composition-matched control diet, or normal chow control diet, from birth for the rd10 mice, and for 5 weeks prior to injury for the PQ-injected mice. Photoreceptor function was analyzed using ERGs and retinal outer nuclear layer (ONL) thickness was measured using optical coherence tomography. Levels of cell survival regulators were analyzed by Western blots on whole retinas.

Results: rd10 mice fed the grape-supplemented diet showed significantly higher rod and cone maximum b-wave amplitudes, indicating elevated photoreceptor responses, when compared with the control diets at each timepoint (N=8, p<0.05). Additionally, implicit times for b-waves were lower in the grape diet (p<0.05), which indicates increased photoreceptor function. The oxidative stress-induced PQ-injected mice on the grape diet also showed significantly higher ERG responses (N=8, p<0.05) and thicker outer nuclear layers (N=8, p<0.05). The retinas from rd10 mice on the grape diet showed significantly lower phospho-GSK3β levels (p<0.05), whereas the oxidative stress-induced mice had significantly higher phospho-GSK3β levels, compared with mice that were on the control diet.

Conclusions: The grape-supplemented diet improved photoreceptor function in two models of retinal degeneration and the mechanism of protection by grapes may involve differential regulation of GSK3β-dependent signaling pathways. Therefore, our study suggests the possibility of grape supplementation as an adjuvant nutritional therapy for the prevention of retinal degenerative diseases.

Commercial Relationships: Maria Esperanza E. Rodriguez Escalante, None; Tinthu Lee, None; Ashley Davis, None; Amit K. Patel, None; Abigail Hackam, None

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Program Number: 4362
Presentation Time: 11:45 AM–12:00 PM
Neuroprotective Effect of Rosiglitazone on Retinal Changes in a Rat Model of Parkinson’s Disease

Eduardo M. Normando1, Ben Davis2, Shereen Nizari2, Lisa Turner2, Giulia Malaguarnera2, Li Guo2, M Francesca Cordeiro1.
1UCL Institute of Ophthalmology & Western Eye Hospital, Imperial College NHS trust, London, United Kingdom; 2Visual Neuroscience, UCL Institute of Ophthalmology, London, United Kingdom.

Purpose: Currently there is an unmet need for early diagnosis and neuroprotective strategies in Parkinson’s Disease (PD). Recently, several studies have also reported that Rosiglitazone (RSG) may be of therapeutic benefit in PD. The aim of this study is to determine whether retinal changes (apoptotic counts and retinal layer thickness) can be used as a biomarker to study effects of systemic treatment in experimental PD.

Methods: Dark Agouti rats (n = 24) were injected daily with rotenone (Rot) or vehicle control for 10 days. Animals were then either left untreated for another 10 days, or treated with daily RSG for 10 days. Retinal Ganglion Cells (RGCs) apoptosis was assessed using DARC and measurements of whole retina, Retinal Nerve Fiber Layer (RNFL), Inner Nuclear Layer (INL) and photoreceptor layer (IS/OS) thickness were obtained simultaneously using SD-OCT. Imaging was performed in vivo at baseline and at 20 days after initial Rot administration. Histology was used to validate in-vivo findings.

Results: Animals treated with Rot revealed a significant increase in RGCs apoptosis after daily Rot administration (64.3 ± 4.6 vs. 33.4 ± 11.5; p<0.001). OCT imaging showed increase in whole retinal thickness (103% ± 2.3% vs. 97.9% ± 1.1%; p<0.01), RNFL (109.5% ± 2.9% vs. 97.4% ± 0.85%; p<0.01), ONL (102.86% ± 1.8% vs. 98.2% ± 0.76%; p<0.05) and IS/OS (106.43% ± 1.3% vs. 99% ± 1.3%; p<0.001) in Rot animals compared to vehicle control. A significant reduction in RGC apoptosis was seen in RSG treated animals (20.7 ± 1.3 spots vs. 68.4 ± 4.6 spots; p<0.001) compared to Rot. A preservation of whole retina (100.6% ± 0.3% vs. 103% ± 2.3%; p<0.01), RNFL (100.6% ± 0.2% vs. 109.3% ± 2.95%; p<0.001) and IS/OS (100.4% ± 0.6% vs. 106.43% ± 1.3%; p<0.001) thickness was also seen in RSG compared to Rot treated animals.

Conclusions: We have shown that RSG is neuroprotective in experimental PD, as shown using retinal imaging with DARC and OCT. This novel finding highlights the potential use of the eye as a window onto the brain, using non-invasive and accessible retinal imaging technology, with important implications for translation to the clinic, where better end point in PD are clearly needed.

Commercial Relationships: Eduardo M. Normando, None; Ben Davis, None; Shereen Nizari, None; Lisa Turner, None; Giulia Malaguarnera, None; Li Guo, None; M Francesca Cordeiro, DARC (P)

Program Number: 4363
Presentation Time: 12:00 PM–12:15 PM
Neuroprotective effects of growth hormone releasing hormone (GHRH) in traumatic optic neuropathy

Manuela Bartolf1, Amany M. Tayfik1, Alan Saul1, Sagar Y. Patel1, David Gay1, Gregory I. Liou1, Sylvia B. Smith1, Julian J. Nussbaum1, Folami Lamokel1.
1Ophthalmology, Georgia Regents University, Augusta, GA; 2Cell Biology and Anatomy, Georgia Regents University, Augusta, GA; 3Biochemistry and Molecular Biology, Augusta, GA; 4University of Texas Southwestern, Dallas, TX.

Purpose: We have previously shown that the hypothalamic secretagogue hormone peptide, growth hormone releasing hormone (GHRH), and its receptors (GHRH-R) are expressed in the retina and that a GHRH agonist prevents diabetes-induced retinal neurovascular injury. Here we have further explored GHRH neuroprotective properties by assessing the effects of the GHRH agonist (JI-34) in a mouse model of traumatic optic neuropathy (TON).

Methods: C57Bl/6j mice were subjected to unilateral optic nerve crush (ONC) injury performed in the left eye, with the contralateral eye serving as a control. A selected group of ONC mice were treated with 5mg/kg of the GHRH agonist JI-34, which received daily subcutaneous injections starting at day 1 post-injury. Both groups...
were subjected to spectral domain optical coherence tomography (SD-OCT) and scotopic threshold response of electroretinography (STR-ERG) to evaluate retinal structural and functional changes, respectively at 7d and 21d post ONC. Immunohistochemical and Western blot analyses were conducted to assess levels of NOGO-A, a marker for axonal regeneration. Retinal ganglion cell (RGC) counting was performed by immunohistochemical analysis of NeuN and Brn3a (RGC markers) on flat mounts of extracted retinas. Specific detection and quantitation of apoptotic cells were done using The DeadEnd™ Fluorometric TUNEL System.

**Results:** GHRH agonist JI-34 prevented retinal ganglion cell loss following ONC at both 7 and 21 days post-injury. SD-OCT analysis showed a preservation of the nerve fiber layer thickness in JI-34 ONC mice. TUNEL assay showed a drastic reduction in the number of detectable apoptotic cells within the inner retina in ONC-mice given the GHRH agonist. Increased NOGO-A immunoreactivity was evident at sites proximal to the lesion and NOGO-A protein levels were increased in ONC injured eyes. GHRH-agonist treated ONC mice, however, displayed a significant decrease in immunopositive areas of NOGO-A as well as a global decrease in protein levels.

**Conclusions:** These results demonstrate GHRH neuroprotective effects in the injured retina and suggest the use of GHRH agonists as novel therapeutic agents for acute and chronic neurodegenerative retinal diseases.

**Commercial Relationships:** Manuela Bartoli, None; Amany M. Tawfik, None; Alan Saul, None; Sagar Y. Patel, None; David Gay, None; Gregory I. Liou, None; Sylvia B. Smith, None; Julian J. Nussbaum, None; Folami Lamoke, None

**Program Number:** 4364

**Presentation Time:** 12:15 PM–12:30 PM

**Protection of pattern electroretinogram (PERG) by oncostatin M after optic nerve injury in mice**

**Rong Wen**, Xin Xia, Tsung-Han Chou, Yiwen Li, Vittorio Porciatti. 1Bascom Palmer Eye Institute, University of Miami, Miami, FL; 2Department of Ophthalmology, Shanghai First Peoples Hospital, Shanghai Jiao Tong University, Shanghai, China.

**Purpose:** Oncostatin M (OSM) is a member of the IL-6 family of cytokines, which includes interleukin 6 (IL-6), IL-11, cliliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), cardiotropin 1 (CT-1), and cardiotrophin-like cytokine (CLC).

We have shown previously that OSM protects rod and cone photoreceptors. The present work investigates the effect of OSM on preserving PERG after optic nerve (ON) crush in mice.

**Methods:** ON-crush was performed unilaterally on 2 months old BALB/C mice. In the treated groups, eyes were injected with recombinant human OSM (3µg in 2µl) or CNTF (3µg in 2µl) immediately after ON crush. The control eyes were injected with 2µl phosphate buffered saline (PBS). PERG was elicited with visual stimuli of contrast-reversing 15 cm. Photopic flash ERGs were also recorded with undilated pupils in response to strobe flashes of 20 cd/m²/s superimposed on a steady background light of 12 cd/m² and presented within a Ganzfeld bowl. Averaged PERG and FERG were analyzed to evaluate the peak-to-trough amplitudes.

**Results:** PERG was recorded before ON crush as baseline and at 8, 15, 22 days after ON-crush. Baseline PERG waveforms and amplitudes in BALB/C mice ranged between 15 and 22 µV. PERG amplitudes of individual mice were normalized to the mean baseline amplitude of each group. Eight days after ON-crush in PBS-treated eyes, PERG amplitude decreased sharply to 15% of the baseline level. In both OSM- and CNTF-treated eyes, however, the PERG amplitudes were significantly higher (P<0.003) than that of the PBS-treated eyes. By day 15, the PERG amplitudes of OSM- and CNTF-treated eyes decreased to the levels close to the noise range, not statistically different from PBS-treated eyes. The PERG amplitudes of all groups were in the noise range 22 days after ON-crush and no significant difference was found among the three groups. No significant changes were observed in photopic flash ERGs over time after ON-crush in any experimental groups.

**Conclusions:** A single injection of OSM or CNTF after ON-crush improves RGC electrophysiological activity. These results provide proof-of-concept for using neurotrophic factors OSM and CNTF for RGC degenerative diseases, including glaucoma and acute optic nerve trauma.

**Commercial Relationships:** Tsung-Han Chou, None; Yiwen Li, None; Vittorio Porciatti, None

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**Program Number:** 4365

**Presentation Time:** 12:30 PM–12:45 PM

**Fractalkine signaling in microglia is neuroprotective in the diabetic retina following lipopolysaccharide-induced activation**

Andrew S. Mendiola, Rolando Garza, Sandra M. Cardona, Aestriz E. Cardona. Biology and STCIEID, The University of Texas at San Antonio, San Antonio, TX.

**Purpose:** Microglia are known to mediate inflammation and neuronal damage during CNS inflammation, however their role in diabetic retinopathy remains unclear. Previous studies from our laboratory showed that CX3CR1 plays neuroprotective roles in models of acute innate immunity and neurodegeneration via inhibition of microglia activation. We hypothesize that in the diabetic retina and brain, absence of CX3CR1 induces rapid activation of microglia leading to pathogenic inflammation via release of inflammatory cytokines and reactive oxygen species potentiating neuronal cell death.

**Methods:** To understand the function of CX3CR1 in diabetes, we crossed the insulin2at2strain (type 1 diabetic mouse model) with our Cx3cr1reporter mice to generate diabetic Cx3cr1+/- (Akita-WT) and diabetic Cx3cr1-/- (Akita-KO) mice. To extend these studies, we challenged 8-10 week old non-diabetic WT or Cx3cr1+/- (HET), Cx3cr1-/- (KO) and Akita-WT and Akita-KO mice with a low dose of lipopolysaccharide (LPS; 20 µg/day) for four consecutive days to establish a low level endotoxemia.

**Results:** Confocal analysis of retinal whole mounts revealed that LPS induced a global hyper-activation and pro-inflammatory phenotype in KO-retinal microglia represented by amoeboid-cellular morphology, round-phagocytic-like cell clusters around blood vessels, and increased iNOS expression when compared to HET-retinas. This phenotype correlated with a significant increase in TUNEL+ neurons and microglia in the retina of KO mice (P<0.05). The degree of microglial activation and inflammation was exacerbated in Akita-KO mice following LPS challenge. Conversely, microglia in Akita-WT retinas resemble naive microglial morphology represented by a branched cellular appearance, few to no round-phagocytic-like cells and no cell clustering. Interestingly, this global activation was also observed in the brain of KO mice, which correlated with a decrease in NeuN+ cells in the visual and lateral cortices, superior colliculus and hippocampus (N=12 mice per genotype).

**Conclusions:** These data support our hypothesis that fractalkine signaling is neuroprotective in the diabetic retina and brain, whereas...
microglia unable to control their effector function following an acute infection, can promote neuronal pathology which can lead to visual impairments.

**Commercial Relationships:** Andrew S. Mendiola, None; Rolando Garza, None; Sandra M. Cardona, None; Astrid E. Cardona, None

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