546 Paradigms of retinal degeneration and rescue
Thursday, May 11, 2017 11:30 AM–1:15 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 5887–5916/B0479–B0508
Organizing Section: Retinal Cell Biology

Program Number: 5887 **Poster Board Number:** B0479
Presentation Time: 11:30 AM–1:15 PM
Retinoschisin is associated with the retinal Na/K-ATPase signaling complex - impact on disease pathology of X-linked juvenile retinoschisis
Karolina A. Ploessl[1], Melanie Royer[1], Sarah Bernklau[1], Neslihan Tavraz[2], Thomas Friedrich[2], Bernhard H. Weber[1], Ulrike Friedrich[1]. [1]Instiute of Human Genetics, University of Regensburg, Regensburg, Germany; [2]Institute of Chemistry, Technical University of Berlin, Berlin, Germany.
Purpose: Mutations in the *RS1* gene cause X-linked juvenile retinoschisis (XLRS), a degenerative disease of the central retina. Still, the molecular processes underlying XLRS pathogenesis are not fully understood. We have recently shown that retinoschisin, the protein encoded by *RS1,* is a regulator of Erk signaling and apoptosis in retinal cells. In this study, we wanted to clarify the role of retinoschisin for the functionality of the Na/K-ATPase, its interaction partner in retinal membranes.
Methods: Retinoschisin binding to specific Na/K-ATPase subunits was investigated in Hek293 cells heterologously expressing different Na/K-ATPase subunit combinations. Ion transport activity of the Na/K-ATPase was analyzed in murine retinal explants from retinoschisin-deficient ($Rs1h^{-/Y}$) mice as well as in *Xenopus leavis* oocytes applying enzymatic assays or atomic absorption spectroscopy, respectively. Signaling was followed in $Rs1h^{-/Y}$ murine retinal explants by immunoblotting analysis for Erk, Ip3/Akt and Ca^{2+} signaling. Co-immunoprecipitation and immunohistochemistry was done to to elucidate intracellular signal transmitters.
Results: Our findings demonstrate that the β2-subunit of the Na/K-ATPase is required for membrane binding of retinoschisin whereas the α-subunit is exchangeable. Our investigation revealed no influence of retinoschisin on ion pump activity or substrate affinity of the retinal Na/K-ATPase. However, we identified an influence of retinoschisin on Na/K-ATPase regulated signaling cascades: Retinoschisin treatment decreased activation of Src and CamKII (markers for Erk and Ca^{2+} signaling) in $Rs1h^{-/Y}$ retinal explants. Co-immmunoprecipitations from murine retinae suggested a direct interaction between retinoschisin, the Na/K-ATPase, and signaling mediators caveolin and Phospholipase C (Plc). Furthermore, we show co-localization of the retinoschisin-Na/K-ATPase complex with intracellular signal tranducers Src, Plc, and the IP3 receptor.
Conclusions: Together, our data suggest that the Na/K-ATPase signalosome complex is reacting to retinoschisin binding. Disturbances in this interaction likely represent an initial step in XLRS pathogenesis.
Commercial Relationships: Karolina A. Ploessl; **Melanie Royer**, None; **Sarah Bernklau**, None; **Neslihan Tavraz**, None; **Thomas Friedrich**, None; **Bernhard H. Weber**, None; **Ulrike Friedrich**, None
Support: FR 3377/1-1 to U.F; Cluster of Excellence "Unifying Concepts in Catalysis" to T.F. and N.T.

Program Number: 5888 **Poster Board Number:** B0480
Presentation Time: 11:30 AM–1:15 PM
Astaxanthin reduces retinal cell death and loss of neuron and glial cell markers in diabetic rats
basma baccouche[1, 2], Sean D. Kim[2], Wei Wei Wang[2], Alistair J. Barber[2]. [1]Ecophysiologie et Procédés Agroalimentaires (EPA), Institut Supérieur de Biotechnologie Sidi Thabet, Université de la Manouba, BiotechPole Sidi Thabet, Tunis, Tunisia; [2]Penn State Hershey Eye Center, Milton S. Hershey Medical Center, Penn State College of Medicine, Hershey, PA.
Purpose: Histological and metabolic changes are major aspects of early retinal degeneration in diabetic retinopathy. Astaxanthin (AST), a dietary carotenoid with potent antioxidant activity, has been suggested to target several health conditions. We investigated whether the administration of AST would protect the rat retina against short-term diabetes-induced damage.
Methods: Two groups of six young male Long-Evans rats were made diabetic by an injection of streptozotocin (STZ, 100 mg/kg, iv). A week after diabetes induction, a group of diabetic and non-diabetic rats were fed 1ml of astaxanthin enriched oil (AstaREAL® L10), containing 10% naturel AST, daily for eight weeks. Age-matched control and diabetic rats were given water. Nine weeks after diabetes induction, animals were sacrificed. One eye of each rat was prefixed, cryosectioned and processed for labeling against the transcription factor Brn3a, glutamine synthetase (GS) and glial fibrillary acid protein (GFAP). Cell death ELISA was performed to determine apoptosis rate on the retina of the second eye. Statistical comparisons were made by one-way ANOVA with a post hoc Tukey's multiple comparisons test (Prism, Graphpad), with $p<0.05$ considered significant.
Results: Diabetes caused a significant decrease in Brn3a expression throughout the neuroretina and was absent from retinal ganglion cell nuclei. GS and GFAP expression were also significantly reduced in STZ-diabetic rat retina compared to normal control animals. AST-treated diabetic rat retina had greater expression of Brn3a in comparison to the untreated diabetic group. In AST-treated controls and diabetic rat retina, astrocytes and Muller cells showed stronger labeling for GFAP and GS positive Muller cell processes were more intense. Cell death ELISA revealed a significant recovery in STZ-diabetic rats treated with AST when compared to untreated diabetic animals ($p<0.05$).
Conclusions: Treatment with the dietary carotenoid, AST, reduced the degeneration of retinal ganglion cells and prevented diabetes-induced retinal cell death in Long-Evans rats. AST also reduced glial cell abnormalities such as the downregulation of GFAP and GS. The results suggest that AST supplementation may provide neuroprotection in the early stages of diabetic retinopathy.
Commercial Relationships: basma baccouche, None; **Sean D. Kim**, None; **Wei Wei Wang**, None; **Alistair J. Barber**, None
Support: The Wood-Whelan Research Fellowships, sponsored by the International Union of Biochemistry and Molecular Biology

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to **http://iovs.arvojournals.org/** to access the versions of record.

Program Number: 5889 **Poster Board Number:** B0481
Presentation Time: 11:30 AM–1:15 PM
Neuroprotective effects of the protein kinase inhibitors, SBJ-051 and sunitinib in light-induced photoreceptor cell death
Byung-Jin Kim[1], Tomohiro Masuda[1], Bhavna J. Antony[2], Derek S. Welsbie[1], Melissa Liu[1], Reji Nair[4], Thomas D. Bannister[4], Jerry L. Prince[2], Timothy P. Spicer[3], Donald J. Zack[1]. [1]The Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD; [2]Electrical and Computer Engineering, Johns Hopkins University, Baltimore, MD; [3]Molecular Therapeutics, The Scripps Research Institute-FL, Jupiter, FL; [4]Chemistry, The Scripps Research Institute-FL, Jupiter, FL.
Purpose: Several kinase pathways have identified as an important modulator on retinal cell survival and functional integrity. Based on our previous finding on multi-kinase inhibitor, sunitinib-mediated RGC protection, we tested potential protective effects of sunitinib and more potent inhibitor, SBJ-051 on photoreceptor (PR) degeneration in the murine light-damage model.
Methods: Female BALB/cJ mice (12wk) were administered with SBJ-051 (10 and 60 mg/kg), sunitinib (15, 30 and 60 mg/kg), or vehicle (5% DMSO) by daily intraperitoneal injection starting 72 h prior to LD up to 6 days post LD. Mice were dark-adapted and insulted with ~3000 lux cool fluorescent light for 4 h followed by immediate dark recovery. Serial SD-OCT imaging, immunohistochemistry, and scotopic ERG were used to assess retinal structure and function. The LD-associated gene expression was profiled using RT-PCR analysis. Phosphorylation of JNK and c-Jun was also assessed from post LD retinas.
Results: Vehicle-treated animals showed a significant decrease (44.78 ± 0.03%, P<0.001) in the retinal outer layer thickness (ROL: OPL to PR) in response to LD. This was also associated with a significant decrease in ERG a-wave (89.99 ± 4.99%, P<0.01) and b-wave (75.61 ± 6.94%, P<0.01) amplitudes. Administration of SBJ-051 at 10 mg/kg and sunitinib at 60 mg/kg markedly attenuated LD-induced ROL thinning up to the latest time point tested. The effect of LD on the ERG a-wave and b-wave amplitudes was also significantly improved with both 10 mg/kg SBJ-051 (a-wave: 67.44 ± 11.93%, b-wave: 58.7 ± 17.65%; P<0.05) and 60 mg/kg sunitinib (a-wave: 62.23 ± 9.54%, b-wave: 65.11 ± 13.43%; P<0.05). Both compounds reduced the loss of rhodopsin and cone arrestin expression with also decreased 5-hydroxy-methyl-cytosine positive cells in the outer nuclear layer. The upregulation of *c-jun*, *c-fos*, *osmr*, *mt2* and *ho1* gene expression at 0 and 3 hours after LD was significantly reduced by 10 mg/kg SBJ-051. SBJ-051 administration also attenuated LD-induced upregulation of retinal JNK and c-Jun phosphorylation (3 h).
Conclusions: Our data demonstrates that SBJ-051 (10 mg/kg) and sunitinib (60 mg/kg) promote PR survival and function following PR damage by LD. We are currently defining the molecular target(s) and mechanisms that mediate the PR neuroprotective activity of these compounds with various approaches.
Commercial Relationships: Byung-Jin Kim, None; **Tomohiro Masuda**, None; **Bhavna J. Antony**, None; **Derek S. Welsbie**, None; **Melissa Liu**, None; **Reji Nair**, None; **Thomas D. Bannister**, None; **Jerry L. Prince**, None; **Timothy P. Spicer**, None; **Donald J. Zack**, None
Support: NEI R01EY001919, NEI P30EY002162 (MML), NEI T32EY007143

Program Number: 5890 **Poster Board Number:** B0482
Presentation Time: 11:30 AM–1:15 PM
Low-to-moderate intensity forced exercise protects retinal function from light-induced retinal degeneration
Lukas Mees[1, 2], Monica Coulter[2], Micah A. Chrenek[1], Cara T. Motz[2], Erica Landis[1], Jeffrey H. Boatright[2, 1], Machelle T. Pardue[2, 3]. [1]Emory University, Atlanta, GA; [2]Atlanta VA Medical Center, Decatur, GA; [3]Georgia Institute of Technology, Atlanta, GA.
Purpose: Exercise has been shown to be protective against light induced retinal degeneration (LIRD) in mice. However, the exercise intensity that produces the most benefit is unknown. We tested the hypothesis that higher treadmill running speeds provide greater protection to retinal function in mice undergoing LIRD.
Methods: Two-month old male BALB/c mice were assigned to one of two control groups (naïve, inactive+LIRD) or three active groups at varying treadmill running speeds for one hour: 5 m/min (low+LIRD), 10 m/min (med+LIRD), and 20 m/min (high+LIRD). Inactive mice were placed on static treadmills. Following two weeks of exercise, mice were either left in maintenance housing light (naïve) or exposed to toxic bright light (10,000 lux; LIRD) for 4 hours. Mice were then exercised for an additional five days, at which point retinal function was assessed by electroretinogram (ERG). Animals were then sacrificed and levels of cathepsin B (CTSB) and brain-derived neurotrophic factor (BDNF) protein were analyzed by enzyme-linked immunosorbent assay (ELISA).
Results: Mice in the low+LIRD or med+LIRD groups had significantly greater bright flash ERG amplitudes compared to inactive+LIRD mice: scotopic a-waves (low+LIRD: 3.8X greater, p=0.004; med+LIRD: 4.48X, p<0.001), scotopic b-waves (low: 3.92X, p<0.001; med: 4.39X, p<0.001), photopic b-waves (low: 3.55X, p<0.001; med: 3.56X, p<0.001) and flicker (low: 3.18X, p=0.005; med: 3.59X, p=0.02). Mice in the high+LIRD group were indistinguishable from inactive+LIRD on all ERG parameters. As expected, naïve mice showed the greatest amplitudes on all waves (p<0.001). CTSB was elevated in active mouse whole brains (p<0.005) but not in retinas, gastrocnemius muscles, or serum. No dosing effect was observed. Retinal BDNF was not elevated with exercise.
Conclusions: Our results show that increased exercise intensity does not provide increased functional protection from LIRD. Interestingly, high+LIRD mice did not show significant benefit. Together this suggests that forced exercise's influence on the retina is sufficient at a low threshold. The molecular basis for this protection is still unclear. It may be that low intensity exercise is sufficient to protect against retinal degeneration, a finding that may have implications for use of exercise as a therapeutic approach in treatment of vision loss.
Commercial Relationships: Lukas Mees, None; **Monica Coulter**, None; **Micah A. Chrenek**, None; **Cara T. Motz**, None; **Erica Landis**, None; **Jeffrey H. Boatright**, None; **Machelle T. Pardue**, None
Support: Dept. of Veteran Affairs facilities and resources; VA RR&D C9246C (Atlanta VA Center of Excellence in Vision and Neurocognitive Rehabilitation); VA RR&D C1924P I21RX001924; Abraham J. & Phyllis Katz Foundation; NEI NIH P30EY006360; NEI NIH R01EY014026; Research to Prevent Blindness, Inc.; Research Career Scientist Award (MTP) from Dept of Veterans Affairs, Rehab R&D Service

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to **http://iovs.arvojournals.org/** to access the versions of record.

Program Number: 5891 **Poster Board Number:** B0483
Presentation Time: 11:30 AM–1:15 PM
Overexpression of spermine oxidase increases neuronal death and glial activation in a model of retinal excitotoxicity
PRAHALATHAN PICHAVARAM[1, 2], Chintan Patel[2, 4], Zhimin Xu[2, 4], Esraa Shosha[2, 4], Manuela Cervelli[3], Ruth B. Caldwell[2, 4], S. Priya Narayanan[4, 1]. [1]College of Allied Health Sciences, Augusta University, Augusta, GA; [2]Vascular Biology Center, Augusta University, Augusta, GA; [3]Department of Science, Roma Tre University, Rome, Italy; [4]Vision Discovery Institute, Augusta University, Augusta, GA.
Purpose: Retinal neuronal injury is a major cause of vision impairment in blinding diseases. Cellular damage resulting from dysregulated polyamine metabolism has been demonstrated to be a major player in neurodegenerative conditions. We have shown that inhibition of polyamine oxidase (PAO) significantly reduces retinal excitotoxicity (Patel et al., ARVO 2016). The aim of this study was to determine the impact of overexpressing spermine oxidase (SMO, a member of PAO family) on this process.
Methods: Cre-LoxP strategy was used to create SMO transgenic mice (SMO Tg) that overexpress SMO in calbindin-positive inner retinal neurons. SMO Tg and WT control mice (10 weeks old) were given intravitreal injections of 20 nmoles NMDA (N-Methyl-D-aspartate) or NMLA (N-Methyl-L-aspartate, control). Animals were sacrificed at various time points (1-7 days) for retinas processed for further analysis.
Results: SMO Tg mice overexpress SMO in horizontal and amacrine cells which are ideally situated to synapse with other retinal neurons to influence neuronal function and survival. The retinas of SMO Tg mice developed normally. NMDA treatment caused significant neuronal death in both SMO Tg and WT mice. However, the loss of NeuN-positive ganglion cells in SMO Tg retina was markedly greater than in their WT controls. ($p<0.01$, N=4-6). The NMDA-induced increase in neuronal death in SMO Tg mice was confirmed by a significant increase in TUNEL positive cells as compared with the WT mice ($p<0.05$ N=3). Morphometry analysis demonstrated significant INL thinning of the NMDA-treated SMO Tg mice compared to WT ($p<0.01$). Increased levels of phosphorylated P-38 in NMDA treated-SMO Tg retinas suggest the involvement stress signaling pathways in SMO mediated neuronal damage. Increased glial activation (studied by Iba1 and GFAP immunostaining, N=4-6) was evident in NMDA-treated SMO Tg retinas, suggesting glial involvement in SMO mediated neuronal damage. Comparison of the NMLA-treated WT and SMO Tg mice retinas showed no differences, indicating that SMO activation is required for its damaging effects.
Conclusions: Our study confirms the critical involvement of SMO in excitotoxicity mediated neuro-glial injury in retina and establishes the SMO Tg mouse as an excellent tool to investigate the cellular and molecular mechanisms involved in retinal neurodegenerative diseases such as diabetic retinopathy and glaucoma.
Commercial Relationships: PRAHALATHAN PICHAVARAM, None; **Chintan Patel**, None; **Zhimin Xu**, None; **Esraa Shosha**, None; **Manuela Cervelli**, None; **Ruth B. Caldwell**, None; **S. Priya Narayanan**, None
Support: Culver VDI Pilot Grant, NEI-R01-EY011766

Program Number: 5892 **Poster Board Number:** B0484
Presentation Time: 11:30 AM–1:15 PM
The Mechanism of Retinal Damage Following Ocular Surface Burn with Alkali
Chengxin Zhou[1, 2], Fengyang Lei[1, 2], Nathan Scott[3], Vassiliki Kapoulea[1, 2], Marie-Claude Robert[4, 5], James Chodosh[6, 2], Claes H. Dohlman[6, 2], Eleftherios I. Paschalis[1, 2]. [1]Department of Ophthalmology, Schepens Eye Research Institute-Massachusetts Eye and Ear, Boston, MA; [2]Harvard Medical School, Boston, MA; [3]Bascom Palmer Eye Institute, University of Miami Health System, Miami, FL; [4]Department of Ophthalmology, Universite de Montreal, Montreal, QC, Canada; [5]Hospitalier de l'Universite de Montreal, Hospital Notre-Dame, Montreal, QC, Canada; [6]Department of Ophthalmology, Massachusetts Eye and Ear, Boston, MA.
Purpose: Alkali burns to the eye constitute a leading cause of worldwide corneal blindness. We recently showed that alkali burns may also cause retinal and optic nerve damage and subsequent irreversible vision loss. This study investigates the physical, biochemical, and immunological components of retinal damage after corneal alkali burns and explores a novel neuroprotective therapy.
Methods: Mice (C57BL/6 and TNF receptor 1/2 knock-out) and Dutch belted pigmented rabbits received central corneal burns using sodium hydroxide. The burned eyes were subsequently irrigated with saline for 15 minutes. Some C57BL/6 mice and rabbits were implanted with pH, redox, and oxygen micro-sensors in the anterior and posterior segments of the eye prior to corneal alkali burn. The *in vivo* measurements were performed for 26 hours. Intraocular pressure (IOP) measurements were performed on mice and rabbits terminally using a microelectromechanical pressure transducer in the anterior chamber. Tissues were collected at different pre-specified time points. Ocular inflammation was assessed with qPCR, *in situ* hybridization, Western Blot, and flow cytometry. Retinal damage and protection was assessed using *in situ* cell death detection assay and immunohistochemistry.
Results: *In vivo* pH, O_2 and redox measurements showed that alkali is effectively buffered by the anterior segment with no direct penetration to the posterior segment. Rather, acute pH, O_2 and redox changes in the anterior chamber caused profound uveal inflammation and subsequent release of pro-inflammatory cytokines posteriorly. In burned eyes, TNF-α mRNA expression was elevated in the cornea and retina- in the latter, particularly in the ganglion cell layer, inner and outer plexiform layers. Deletion of TNF receptor genes in burned mice suppressed retinal inflammation and retinal damage. Likewise, prompt TNF-α inhibition by monoclonal antibody also suppressed retinal inflammation and damage in mice and rabbits.
Conclusions: This study suggests that corneal alkali burns cause retinal damage via secondary inflammation and upregulation of TNF-α expression. Alkali is well buffered by the anterior segment. Prompt TNF-α inhibition substantially protects the eye and this finding may be clinically important for the treatment of ocular burns.
Commercial Relationships: Chengxin Zhou, None; **Fengyang Lei**, None; **Nathan Scott**, None; **Vassiliki Kapoulea**, None; **Marie-Claude Robert**, None; **James Chodosh**, None; **Claes H. Dohlman**, None; **Eleftherios I. Paschalis**, None
Support: Boston keratoprosthesis Research Fund, Massachusetts Eye and Ear, Boston, MA

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to **http://iovs.arvojournals.org/** to access the versions of record.

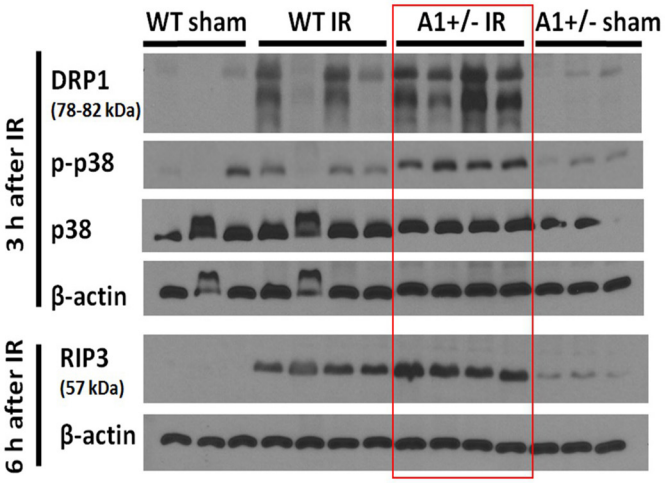
Program Number: 5893 **Poster Board Number:** B0485
Presentation Time: 11:30 AM–1:15 PM
Amelioration of Visual Deficits and Pathology after Mild TBI with the CB2 Inverse Agonist SMM189
Anton Reiner[1], Natalie Guley[1], Nobel Del Mar[1], Bob M. Moore[2], Marcia G. Honig[1]. [1]Dept of Anatomy & Neurobiology, Univ of Tennessee Health Sci Ctr, Memphis, TN; [2]Pharmaceutical Sciences, Univ of Tennessee Health SCi Ctr, Memphis, TN.
Purpose: Mild TBI is often accompanied by visual system dysfunction and injury, which is at least in part caused by the microglial neuroinflammatory process initiated by the injury. Using our focal cranial blast mouse model of closed-skull mild TBI, we evaluated the ability of the CB2 inverse agonist SMM189, which biases microglia from the harmful M1 state to the beneficial M2 state, to mitigate visual system dysfunction and injury after TBI.
Methods: Male C57BL/6 or Thy1-EYFP reporter mice received closed-head blast of 0-psi (sham) or 50-psi to the left side of the cranium, as previously described (Guley et al., JNeurotrauma 2016). Blast mice received daily vehicle (50V) or 6 mg/kg SMM189 (50SMM) for two weeks beginning 2 hours after blast. Sham mice received vehicle (0V). Mice were functionally assessed by their scotopic ERG and by Optomotry 30 days after blast, and then by OCT. Retina and optic nerve/tract were assessed morphologically 3-7 days or 30-80 days after blast.
Results: Contrast sensitivity in 50V mice was significantly reduced in both eyes at 30 days post-blast, but rescued in 50SMM mice. The left eye (LE) ERG B-wave peak was also abnormal in 50V mice, and SMM189 treatment restored it to control levels. OCT at 30 days post-blast revealed outer retinal thinning in the LE of 50V mice, but not in 50SMM mice. Immunolabeling showed a significant increase in microglia in the LE of 50V mice at 3 days post-blast, and a lesser elevation in both eyes at 30 days post-blast. SMM189 decreased the microglial elevation at both 3 and 30 days. GFAP immunolabeling of Müller cells was also elevated in the LE of 50V animals at 30 days, which was normalized by SMM189. Analysis of optic tract microglia for the M1 versus M2 markers CD16/32 and CD206 revealed that SMM189 biased microglia toward the M2 state. Analysis of left optic nerve 11 weeks post-blast revealed axon loss in 50V animals and rescue in 50SMM mice. At 3 days post-blast, axon damage was seen in the continuation of the left optic nerve (i.e. the right optic tract) in the form of swollen axon bulbs in 50V mice, but SMM189 treatment significantly reduced their abundance.
Conclusions: The novel drug SMM189 significantly mitigated visual system dysfunction and pathology caused by mild TBI. Our findings suggest the value of combatting visual system injury after TBI by using CB2 inverse agonists to target microglia and bias them toward the M2 state.
Commercial Relationships: Anton Reiner, None; **Natalie Guley**, None; **Nobel Del Mar**, None; **Bob M. Moore**, None; **Marcia G. Honig**, None
Support: The Methodist Hospitals Professor of Neuroscience (AR), RO1EY05298 (AR), The Graduate College of The University of Tennessee Health Science Center (NMG), and The University of Tennessee Neuroscience Institute (AR, NMG)

Program Number: 5894 **Poster Board Number:** B0486
Presentation Time: 11:30 AM–1:15 PM
Toll-like receptor 2 promotes photoreceptor survival in acute retinal injury
Marcus Hooper, Clayton P. Santiago, Raghav Ramchander, John D. Ash. Ophthalmology, University of Florida, Gainesville, FL.
Purpose: Retinal stress can induce the expression of leukemia inhibitory factor (LIF), which protects photoreceptors from cell death. The mechanism of LIF induction has not been identified. Toll-like receptor 2 (TLR2) is a pattern recognition receptor that is activated by damage-associated molecular patterns (DAMPS). Injection of the TLR2 agonist PAM3CSK, has been shown to induce LIF expression in the retina which protects photoreceptors from light damage. In this project we use TLR2$^{-/-}$ mice to investigate the role of TLR2 in LIF induction and protection from light damage (LD). NF-kB is a transcription factor that is activated by TLR2 signaling.
Methods: All mice were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. For LD, TLR2$^{-/-}$ and wild type (WT) mice were exposed to ≥ 1300 lux for 4 hours. Retinal photoreceptor function was measured using scotopic electroretinography (ERG). Retinal morphology and Outer nuclear layer (ONL) thickness were examined using Spectral Domain-Optical Coherence Tomography (OCT), and histology. Quantitative PCR was used to measure gene expression. Chromatin immunoprecipitation was used to examine NF-kB binding sites in the LIF gene.
Results: Exposure to damaging light in WT mice results in elevated expression of LIF. LIF induction correlated with NF-kB binding within a chromatin region located in the first intron of the LIF gene, that we show is a potentent transcriptional enhancer of the LIF promoter. TLR2$^{-/-}$ mice failed to induce LIF expression following exposure to damageing light. Interestingly, male TLR2$^{-/-}$ mice exposed to damaging light have a greater reduction in ONL thickness than WT mice, and have a greater reduction in ERG a-waves. However, female TLR2$^{-/-}$ mice were similar to WT mice.
Conclusions: Our data show that induction of LIF can be mediated by TLR2 and NF-kB activation, and suggests that stressed photoreceptors activate TLR2 by release of DAMPS. The failed induction of LIF in TLR2$^{-/-}$ mice reduced photoreceptor survival in male mice but not female mice. The increase in sensitivity to LD is likely due to a concomitant deficiency in LIF induction in TLR2$^{-/-}$ mice. We do not know why males are more affected than females, but we have identified estrogen receptor binding sites in the LIF promoter as well, suggesting that there are multiple pathways that can induce LIF expression.
Commercial Relationships: Marcus Hooper, None; **Clayton P. Santiago**, None; **Raghav Ramchander**; **John D. Ash**, None
Support: Funding support to JDA include NIH R01EY016459-11, foundation fighting blindness, and an unrestricted departmental grant from Research to Prevent Blindness inc. Training support include training grant EY007132.

Program Number: 5895 **Poster Board Number:** B0487
Presentation Time: 11:30 AM–1:15 PM
Protective Role of Arginase 1 in Retinal Ischemia Reperfusion Injury
Abdelrahman Fouda[1], Zhimin Xu[1], Esraa Shosha[1], William Caldwell[2], S. Priya Narayanan[1], Ruth B. Caldwell[1]. [1]Vascular Biology Center, Augusta University, AUGUSTA, GA; [2]Pharmacology, Augusta University, Augusta, GA.
Purpose: Studies from our lab and others have demonstrated the involvement of the arginase enzyme in different neurovascular diseases. Arginase competes with nitric oxide synthase (NOS) enzymes in endothelial cells (eNOS) leading to endothelial dysfunction and in myeloid cells (iNOS) thus promoting a reparative phenotype. We have recently shown that the mitochondrial isoform, arginase 2 (A2), has a key role in retinal ischemia/reperfusion (IR) injury. Here we aimed to examine the role of the cytosolic isoform A1 in retinal IR injury.

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to **http://iovs.arvojournals.org/** to access the versions of record.

Methods: Wild type (WT), A1$^{+/-}$ knock out (KO), endothelial (Cdh5-cre;A1$^{f/f}$) KO, and myeloid specific (LysM-cre;A1$^{f/f}$) KO mice were subjected to retinal IR (40 min, right eye - left eye served as sham control). A cohort of WT mice were treated with intravitreal injection of pegylated A1 or PBS 3h before IR. Another cohort of WT mice received clodronate or control liposomes (200 uL, i.p., 1 d before and 3 d after IR) to deplete systemic monocytes/macrophages. Retinas were collected at different time points for analysis. Neurodegeneration and microglia/macrophage activation were analyzed at 7 d using NeuN and Iba-1 flat-mount staining followed by confocal microscopy. Vascular injury was assessed by acellular capillary formation. Necrotic cell death was studied by propidium iodide (PI) labelling and western blot for RIP3.
Results: A1 deletion exacerbated IR-induced neuronal and vascular injury as measured by reduced NeuN positive cells and increased acellular capillaries by 50 % ($p<0.05$, n=4-6). Furthermore, A1$^{+/-}$ mice showed reduced retinal thickness (H&E stain) by 5% ($p<0.05$, n=4-5) and increased necroptosis demonstrated by a 70% increase in the number of PI+ cells ($p<0.05$, n=5) as well as increased RIP3 expression at 6 h after IR. Western blotting showed increased levels of the mitochondrial fission protein, Drp-1, and the stress marker p-p38 in A1$^{+/-}$ mice compared to WT 3 h after IR (figure). Treatment with peg A1 restored neuron survival in WT mice, whereas macrophage depletion with clodronate liposomes increased neuron loss. Cell specific deletion of A1 in myeloid cells showed exacerbated neuron loss but deletion of A1 in endothelial cell had no effect (table).
Conclusions: In contrast to A2, A1 is neurovascular protective in retinal IR via decreasing necroptosis and mitochondrial dysfunction. Myeloid derived A1 could play a role in mediating a protective effect of macrophages after retinal IR.



Mice subjected to IR and sacrificed at 7d	N=	% Neuronal Survival (Mean±SD)	Statistics (t-test or one-way ANOVA)	Interpretation
A1$^{+/-}$ global KO	3	46.2±9.2	*p<0.05 vs WT	A1 deletion exacerbates neurodegeneration
WT (C57BL6J)	6	66.2±3.9		
WT+Peg A1 0.6 ng	1	70		A1 upregulation ameliorates neurodegeneration
WT+Peg A1 1.7 ng	5	81.1±6.3	*p<0.05 vs WT+PBS	
WT+Peg A1 3.4 ng	5	75.8±18.2		
WT+Peg A1 6.8 ng	5	57.9±18.1		
WT+PBS	6	64.1±11.6		
LysM-cre;A1$^{f/f}$	4	52.6±9.1	*p<0.05 vs A1$^{f/f}$	Myeloid but not endothelial A1 mediates neuroprotection
Cdh5-cre;A1$^{f/f}$	3	65.8±5.5		
A1$^{f/f}$	7	66.1±9.7		
WT+Clodronate liposomes	3	40±6.2	*p<0.05 vs WT+Ctrl lipo	Systemic macrophage depletion exacerbates neurodegeneration
WT+Ctrl liposomes	3	57.9±8.8		

Commercial Relationships: Abdelrahman Fouda, None; **Zhimin Xu**, None; **Esraa Shosha**, None; **William Caldwell**, None; **S. Priya Narayanan**, None; **Ruth B. Caldwell**, None
Support: NEI-EY11766, VA Merit Award, AHA-11SDG7440088

Program Number: 5896 **Poster Board Number:** B0488
Presentation Time: 11:30 AM–1:15 PM

K-ATP channels protect the retina at critical early stages of ischemia

Andrey V. Dmitriev[1], *Robert A. Linsenmeier*[1, 2]. [1]Department of Biomedical Engineering, Northwestern University, Evanston, IL; [2]Neurobiology Departament, Northwestern University, Evanston, IL.

Purpose: To investigate the role of K-ATP channels in stabilization of ionic homeostasis at earlier stages of ischemia.
Methods: To characterize the disturbances of ionic homeostasis during ischemia we measured local electrical responses to light (LERG) and both steady state levels and light-induced changes of extracellular concentrations of K^+ and Ca^{++} in the isolated mouse retina using appropriate microelectrodes. An oxygen-glucose deprivation protocol (OGD) was used to mimic ischemia, and glybenclamide (0.1 mM) was applied to block K-ATP channels.
Results: 30 minutes of OGD reversibly suppressed the light-induced activity of the retina. The bipolar cell-dependent b-wave of the ERG started to decline already 1 minute after the onset of OGD and completely disappeared in 4-5 minutes. The photoreceptor-dependent slow PIII component of the ERG was less sensitive, but also was suppressed after 15 – 20 minutes. Similarly, the light-induced increase of $[K^+]_o$ in the inner retina disappeared in 5 minutes, while the decrease of $[K^+]_o$ in the photoreceptor layer diminished, but still persisted for at least 20 minutes. Right after OGD ended all these responses began to recover, and after 30 minutes their amplitudes were close to the pre-ischemic values, with the exception of the b-wave, which only partly recovered. OGD also evoked reversible changes in ionic concentration: an increase of $[K^+]_o$ and a decrease of $[Ca^{++}]_o$. These concentration changes were expected, but their rather modest amplitudes (up to 10 – 12 mM for $[K^+]_o$ and 0.8 mM for $[Ca^{++}]_o$) were surprising. The changes were much more dramatic when the K-ATP channel blocker glybenclamide was applied during OGD. In the inner retina $[K^+]_o$ increased more than 10 times (up to 40 mM) and $[Ca^{++}]_o$ decreased about 4 times (down to 0.3 mM). The recovery rate after OGD with glybenclamide was significantly reduced compared to recovery after OGD alone.
Conclusions: 1) Ischemia disturbs first the ability of the retina to respond to light. But the retina can recover if ischemia does not reach the point of failure of ionic homeostasis. 2) K-ATP channels play a role in natural protection from ischemia, delaying the collapse of ionic homeostasis. Blocking K-ATP channels disarms the retina and leads to catastrophic ionic disturbances with unrepairable consequences. 3) A promising strategy against ischemia might be the mobilization of K-ATP channel based defense, possibly using preconditioning.
Commercial Relationships: Andrey V. Dmitriev, None; **Robert A. Linsenmeier**, None

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to **http://iovs.arvojournals.org/** to access the versions of record.

Program Number: 5897 **Poster Board Number:** B0489
Presentation Time: 11:30 AM–1:15 PM
Enriched environment decreases retinal susceptibility to ischemic damage
Maria Florencia Gonzalez Fleitas, Damian Dorfman, Marcos Luis Aranda, Hernan Hugo Dieguez, Pablo Sande, Monica Chianelli, Ruth E. Rosenstein. Human Biochemistry, School of Medicine, University of Buenos Aires, CEFyBO/CONICET, Retinal Neurochemistry and Experimental Ophthalmology Laboratory, Buenos Aires, Argentina.
Purpose:
Ischemia is a key component of several retinal diseases that are leading causes of irreversible blindness. At present, there are no effective strategies to prevent retinal ischemic damage. Enriched environment (EE) refers to conditions that facilitate or enhance sensory, cognitive, motor, and social stimulation relative to standard (laboratory) conditions. The aim of the present work was to analyze whether the exposure to EE prevents acute retinal ischemic damage.
Methods:
Adult male *Wistar* rats were housed instandard environment (SE) or EE for 3 weeks. SE consisted in standard laboratory cages, housing 2 animals/cage, and EE consisted in big cages housing 6 animals and containing several food hoppers, wheels and different objects repositioned once/day and fully substituted once/week. In SE and EE, animals were provided with food and water *ad libitum*. After 3 weeks of SE or EE, retinal ischemia was induced by increasing intraocular pressure above120 mm Hg for 40 min.
Immediately after ischemia, both groups were further housed in SE for 2 weeks. Retinal function (electroretinography, ERG), histology (H&E), Muller cells (glial fibrillary acidic protein, (GFAP immunoreactivity)) and retinal ganglion cell (RGC) number (Brn3a immunohistochemistry) were assessed.
Results:
In animals housed in SE, ischemia induced a significant decrease in ERG a- and b- wave amplitude and oscillatory potentials, whereas the previous exposure to EE prevented these functional alterations. Two weeks after ischemia, a significant decrease in the total retinal thickness and the number of Brn3a(+) cells, as well as an increase in Muller cell GFAP levels werefound in retinas from animals housed in SE, whereas in ischemic retinas from animals housed in EE, theseparameters were significantly preserved.
Conclusions:
These results suggest that the exposure to EE prevents retinal alterations induced by acute ischemia.
Commercial Relationships: Maria Florencia Gonzalez Fleitas, None; **Damian Dorfman**, None; **Marcos Luis Aranda**, None; **Hernan Hugo Dieguez**, None; **Pablo Sande**, None; **Monica Chianelli**, None; **Ruth E. Rosenstein**, None

Program Number: 5898 **Poster Board Number:** B0490
Presentation Time: 11:30 AM–1:15 PM
Neuroprotective evaluation of topically delivered trabodenoson in an acute ischemic rodent model
James A. Gow[1], Craig E. Crosson[2], David Albers[1], Cadmus C. Rich[1], Rudolf Baumgartner[1]. [1]Inotek Pharmaceuticals, Lexington, MA; [2]Ophthalmology, Medical University of South Carolina, Charleston, SC.
Purpose: Trabodenoson, an adenosine mimetic highly selective for the A_1 receptor, has been reported to lower IOP in humans with glaucoma (Myers et al (2016) JOPT 32, 555). As the stimulation of adenosine A_1 receptors has been shown to be neuroprotective in several systems, we sought to evaluate whether topical (ocular) delivery of trabodenoson can protect the retina from acute ischemic injury.
Methods: Male Brown Norway rats received unilateral ischemic injury (IOP elevated to 160 mmHg for 45 min) on Day 0. The contralateral eye from each animal served as control. Full field scotopic ERGs were recorded on the day prior to ischemia (Day -1) and 7 days post-ischemic injury. Treated animals received ocular (topical) drops of 6% trabodenoson (5 ml) bilaterally starting 2 hrs prior to ischemic injury (Day 0), and then twice daily on Study Days 1 through 7. Treated animal were compared to untreated control animals.
Results: In control animals, ERG analysis of ischemic eyes 7 days post-injury demonstrated that mean a- and b-wave amplitudes were significantly reduced from baseline values by 49 ± 6% and 60 ± 7%, respectively. In trabodenoson-treated animals, ERG analysis of ischemic eyes demonstrated that mean a- and b-wave amplitudes were reduced from baseline values by 29 ± 4% and 30 ± 5%, respectively. Comparing corresponding eyes from control and trabodenoson-treated animals revealed that trabodenoson treatment significantly ($p<0.05$) preserved inner retinal function as measured by increased b-wave amplitude. A trend toward preservation of photoreceptor function was measured as an increase in a-wave amplitude. No significant changes from baseline a- and b-wave amplitudes in contralateral, non-ischemic eyes from trabodenoson-treated or control animals were measured.
Conclusions: These initial results support the idea that topical (ocular) delivery of trabodenoson can protect the retina from ischemic injury. This retinal neuroprotection was primarily associated with preserving inner retinal function. Additional studies should be conducted to further validate trabodenoson's retinal neuroprotective activity.
Commercial Relationships: James A. Gow, Inotek Pharmaceuticals (E); **Craig E. Crosson**, Inotek Pharmaceuticals (C); **David Albers**, Inotek Pharmaceuticals (E); **Cadmus C. Rich**, Inotek Pharmaceuticals (E); **Rudolf Baumgartner**, Inotek Pharmaceuticals (E)
Support: Inotek Pharmaceuticals

Program Number: 5899 **Poster Board Number:** B0491
Presentation Time: 11:30 AM–1:15 PM
Retinal Ischemia Reperfusion: Role of Arginase 2
Esraa Shosha[1, 2], Abdelrahman Fouda[1, 2], Ahmed Ibrahim[3], Mohamed A. Al-Shabrawey[3], Zhimin Xu[1, 2], Tahira Lemtalsi[1, 2], William Caldwell[4], S. Priya Narayanan[5, 2], Ruth B. Caldwell[1, 2]. [1]Vascualr Biology Center, Augusta University, Augusta, GA; [2]Vision Discovery Institute, Augusta University, Augusta, GA; [3]Oral Biology, Augusta University, Augusta, GA; [4]Pharmacology and Toxicology, Augusta University, Augusta, GA; [5]College of Allied Health Sciences, Augusta University, Augusta, GA.
Purpose: We have previously shown the involvement of the mitochondrial enzyme arginase 2 (A2) in inducing neurovascular degeneration in retinal ischemia reperfusion (I/R) injury (Shosha et al, 2016). A2 deletion significantly protected against neurovascular degeneration, retinal thinning and cell death. The present study was undertaken to determine the role of A2 in I/R induced retinal detachment, hyperpermeability and mitochondrial dynamics and to model the I/R injury *in vitro*.
Methods: We used mice lacking both copies of arginase 2 (A2-/-) and wild type (WT) controls. I/R insult was conducted on the right eye and the left eye was used as control. Optical coherence tomography (OCT) imaging was used to evaluate retinal detachment. Western blot was used to assess vascular permeability by measuring albumin extravasation and to evaluate mitochondrial injury by

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to **http://iovs.arvojournals.org/** to access the versions of record.

measuring the mitochondrial fission marker dynamin related protein 1 (Drp1). Oxygen glucose deprivation/reperfusion (OGD/R) was used to simulate I/R injury in bovine retinal endothelial (BRE) cells *in vitro*. A2 expression in relation to cell death and survival pathways were evaluated by western blot.

Results:

OCT showed a marked distortion of retinal layers, retinal detachment and edema in WT retinas at 1 week after I/R injury (n=5). A2-/- I/R retinas (n=4) showed more preserved retinal structure, less retinal detachment and edema, and more normal fundus compared to WT I/R. At 5 weeks post injury, the retinal detachment and edema were resolved in all mice. I/R injury caused a prominent extravasation of mouse albumin at 2 days post injury ($P<0.001$, n=4). This was significantly reduced in the A2-/- I/R retinas ($P<0.05$, n=4). Levels of Drp1 were significantly increased at 3h after I/R in WT retinas ($P<0.05$, n=3). This I/R-induced increase in Drp1 was prevented by A2 deletion (n=3). For OGD/R studies, we found an increase in A2 expression after 6h OGD followed by 6h reperfusion (n=3). The A2 increase was associated with increases in phosphorylation of the stress marker P38 ($P<0.01$, n=3) and reduced phosphorylation of the survival marker AKT (n=2) along with increases in the cell death marker cleaved poly ADP-ribose polymerase (PARP) ($P<0.01$, n=3).

Conclusions:

This study shows for the first time A2 deletion protects against retinal detachment and vascular permeability increase after I/R injury through a mechanism associated with mitochondrial fission.

Commercial Relationships: Esraa Shosha; **Abdelrahman Fouda**, None; **Ahmed Ibrahim**, None; **Mohamed A. Al-Shabrawey**, None; **Zhimin Xu**, None; **Tahira Lemtalsi**, None; **William Caldwell**, None; **S. Priya Narayanan**, None; **Ruth B. Caldwell**, None

Support: NEI-EY11766, VA Merit Award, AHA-11SDG7440088, AHA-15PRE25560007

Program Number: 5900 **Poster Board Number:** B0492
Presentation Time: 11:30 AM–1:15 PM

Daily Zeaxanthin Supplementation Improves RPE Function in a Mouse Model of RPE Oxidative Stress

Manas Ranjan Biswal[1], Brad Justis[1], Ping Yang Han[1], Hong Li[1], C Kathleen Dorey[2], Dennis L. Gierhart[3], Alfred S. Lewin[1]. [1]Molecular Genetics and Microbiology, University of Florida College of Medicine, Gainesville, FL; [2]Biomedical Science, Virginia Tech Carilion School of Medicine, Roanoke, VA; [3]Research and Development, ZeaVision LLC, Chesterfield, MO.

Purpose: The purpose of the study is to test the hypothesis that zeaxanthin supplementation would prevent on oxidative damage and pathology in the retinal pigment epithelium (RPE) in *Sod2*$^{flox/flox}$*-VMD2-cre* mice. RPE-specific deletion of *Sod2* (manganese superoxide dismutase) in this mice model causes signs of atrophy characteristic of dry age-related macular degeneration (AMD).

Methods: Zeaxanthin beadlets were dissolved in sterile water (1gm/kg body weight). For the experimental group (n=10), diluted Zeaxanthin beadlets were delivered by daily gavage to mice starting at one month of age and extending up to five months of age. A group of mice (n=8) of same ages not receiving any supplementation served as a control. Zeaxanthin retention in the retina/RPE and liver was quantified one month following supplementation. Retinal function was evaluated by electroretinogram (ERG) and optical coherence tomography (OCT). Quantitative real time PCR of antioxidant genes was used to measure the levels of antioxidant genes after one month and four months of zeaxanthin supplementation.

Results: Zeaxanthin supplementation led to 5-fold increases in zeaxanthin in the retina/RPE (12.8ng/gram tissue) and 12-fold increases in liver (205ng/ gram tissue) compared to control mice. There was a significant decrease in a-wave and b-wave in both control and zeaxanthin-treated mice control mice by five months of age. However, we saw improved c-wave ERG amplitude (28%; $p<0.01$) and thicker RPE ($p<0.01$) in experimental mice than control mice suggesting improved function of RPE. After one month of zeaxanthin supplementation, we did not find any changes in antioxidant gene (CAT, GSTM1, HO1, NQO1, and SQSTM1) expression in the RPE/choroid, but after 4 months of supplementation there was a significant increase in expression of the same genes.

Conclusions: We conclude that daily supplementation of zeaxanthin leads to increased accumulation of this carotenoid in the retina/RPE, induction of antioxidant enzymes, and improved structure and function of the RPE.

Commercial Relationships: Manas Ranjan Biswal, None; **Brad Justis**, None; **Ping Yang Han**, None; **Hong Li**, None; **C Kathleen Dorey**, None; **Dennis L. Gierhart**, ZeaVision LLC (F); **Alfred S. Lewin**, None

Support: Shaler Richardson Professorship endowment, ZeaVision LLC, National Eye Institute Grant 1K99EY027013

Program Number: 5901 **Poster Board Number:** B0493
Presentation Time: 11:30 AM–1:15 PM

Tamoxifen provides structural and functional rescue in murine models of photoreceptor degeneration

Xu Wang[1], Lian Zhao[1], Wenxin Ma[1], Yikui Zhang[1], Shaimar Gonzalez[1], Jianguo Fan[2], Friedrich Kretschmer[3], Tudor C. Badea[3], Haohua Qian[4], Wai T. Wong[1]. [1]Unit on Neuron-Glia Interactions in Retinal Disease, National Eye Institute, National Institutes of Health, Bethesda, MD; [2]Section on Molecular Structure and Functional Genomics, National Eye Institute, National Institutes of Health, Bethesda, MD; [3]Retinal Circuit Development and Genetics Unit, National Eye Institute, National Institutes of Health, Bethesda, MD; [4]Visual Function Core, National Eye Institute, National Institutes of Health, Bethesda, MD.

Purpose: Photoreceptor (PR) degeneration is a cause of irreversible vision loss in incurable blinding retinal diseases including retinitis pigmentosa (RP) and atrophic age-related macular degeneration. Recent studies have implicated non-cell autonomous contributions from microglia to the rate of PR degeneration. We investigate tamoxifen, a selective estrogen receptor modulator (SERM), as an agent to ameliorate PR degeneration via the inhibition of microglial activation.

Methods: We evaluated the effects of oral tamoxifen administration (80mg/kg daily) in: (1) a light-induced PR injury model, and (2) the rd10 (*Pde6b*^{rd10}) genetic model for RP. Evaluations performed include *in vivo* structural imaging (OCT and autofluorescence) and functional testing (ERG and optokinetic response (OKR) measurement), and immunohistological analyses. Cytokine analyses were performed using a Milliplex assay kit. *In vitro* studies examining microglia-PR interactions were performed.

Results: We found that tamoxifen, a drug previously linked with retinal toxicity, paradoxically provided potent neuroprotective effects in the context of retinal injury. In a light-induced degeneration model, tamoxifen prevented onset of photoreceptor apoptosis and atrophy, and maintained near-normal levels of electroretinographic responses. Rescue effects were correlated with decreased microglial activation and inflammatory cytokine production in the retina *in vivo*, and a reduction of microglia-mediated toxicity to photoreceptors *in vitro*, indicating a microglia-mediated mechanism of rescue. Tamoxifen also attenuated degeneration in a genetic (*Pde6b*^{rd10}) model of retinitis pigmentosa, significantly improving retinal structure, electrophysiological responses, and visual behavior.

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to **http://iovs.arvojournals.org/** to access the versions of record.

Conclusions: Tamoxifen exerts prominent neuroprotective effects in two separate mouse models of PR degeneration. Further studies into tamoxifen as a drug suitable for being repurposed to treat PR degeneration in retinal disease are warranted.
Commercial Relationships: Xu Wang, U.S. Patent Application No. 62/377,439, (P); **Lian Zhao**, U.S. Patent Application No. 62/377,439, (P); **Wenxin Ma**, U.S. Patent Application No. 62/377,439, (P); **Yikui Zhang**, None; **Shaimar Gonzalez**, None; **Jianguo Fan**, None; **Friedrich Kretschmer**, None; **Tudor C. Badea**, None; **Haohua Qian**, None; **Wai T. Wong**, U.S. Patent Application No. 62/377,439, (P)
Support: funds from the National Eye Institute Intramural Research Program.

Program Number: 5902 **Poster Board Number:** B0494
Presentation Time: 11:30 AM–1:15 PM
Using RiboTag and Sun1-sfGFP technologies to map Müller cell-specific epigenetic regulation of stress-induced LIF expression in vivo
Clayton P. Santiago, Casey J. Keuthan, Aisha A. Imam, John D. Ash. Ophthalmology, University of Florida, Gainesville, FL.
Purpose: Stressed photoreceptors release signals that stimulate Müller glial cells to induce the expression of trophic factors such as Leukemia Inhibitory Factor (LIF). Understanding the regulation of LIF expression is key to elucidating the mechanisms that control the rate of retinal degeneration. We have previously shown epigenetic regulation of the LIF locus in whole retinal samples. Since Müller glial cells make up approximately only 5% of the retinal cell population, the presence of other retinal cell types may have masked certain regulatory elements. The aim of this study is to confirm the cell type expression of LIF as well as the epigenetic profile of the LIF locus specifically in Müller glial cells.
Methods: All procedures with animals were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Müller glia-specific (GLAST Cre) and neural retina (Chx10 Cre) with either RPL22:HA$^{f/+}$ or Sun1-sfGFP$^{f/+}$ mice were injected with TNFα to induce LIF expression. PBS-injected animals were used as control. For RNA analysis, the retinas from RPL22:HA$^{f/+}$ animals were homogenized, polysome-associated RNA was precipitated by αHA, and RNA was purified for analysis by qRT-PCR. For chromatin analysis, the retinas were fixed and homogenized. Intact nuclei were precipitated by αMyc, fragmented with MNase and prepared for chromatin immunoprecipitation (ChIP). Antibodies against NF-κB, H3K4me3, H3K27me3 or IgG were used. DNA was then purified for analysis by qPCR.
Results: qRT-PCR analysis shows that LIF expression is enriched in Müller cells while other stress markers such as the photoreceptor-specific endothelin-2 is not. ChIP analysis against H3K27me3 and NF-κB denote the presence of poised enhancer-like elements within the first intron of the LIF gene. TNFα-injected mice exhibit increased promoter-associated H3K4me3 levels within the internal transcription start site of the LIF gene in both Müller cells as well as neural retina. Interestingly, this mark is increased at the distal transcription start site in Müller cells, but is masked in samples from the neural retina.
Conclusions: Our data confirms that Müller cells express LIF. We also show that majority of the retinal epigenetic changes taking place at the LIF genomic locus originates from the Müller cells.
Commercial Relationships: Clayton P. Santiago, None; **Casey J. Keuthan**, None; **Aisha A. Imam**, None; **John D. Ash**, None
Support: Funding support to John Ash include NIH R01EY016459-11, Foundation Fighting Blindness, and an unrestricted departmental grant from Research to Prevent Blindness, Inc.

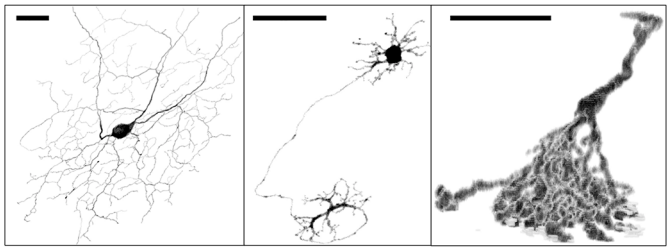
Program Number: 5903 **Poster Board Number:** B0495
Presentation Time: 11:30 AM–1:15 PM
Screen for Small Molecules that Promote Myelination of Human Stem Cell Derived Oligodenrocytes
xitiz chamling[1], Valentin Sluch[2], Karl J. Wahlin[3], Donald J. Zack[1]. [1]Ophthalmology, Wilmer Eye Institute, Baltimore, MD; [2]Novartis, Boston, MA; [3]Univeristy of San Diego, San Diego, CA.
Purpose: Multiple Sclerosis, a neurodegenerative disease whose pathogenesis involves immune-mediated demyelination, is a leading cause of optic neuritis. Currently, there are no clinically approved therapeutic approaches that directly promote neuronal remyelination and/or survival. As an approach to developing such therapies, we are working to establish an *in vitro* myelination assay that works with human cell culture systems, with the goal of performing a high-content screen of small molecule libraries to identify lead molecules that promote myelination, and thereby help to develop a novel strategy to complement current approaches for MS treatment.
Methods: We used CRISPR/Cas9 genome editing to generate knock-in reporter stem cell lines for OPCs and oligodendrocytes (OLs). After differentiation the reporter OPCs can be purified using either FACS sorting or immunopurifcation. We have successfully used this strategy to develop reporters for stem cell-derived RGC differentiation and purification. We will then co-culture purified RGCs and purified OPCs to establish an in-vitro myelination system. Either the co-culture or the immunopurified OPCs will be plated into a 384-well or a 1536-well culture format for high-throughput screening. An Echo (Labcyte) acoustic liquid handler will be used to aliquot library compounds, and a tip-based automated robotic liquid handling system will be used to add other reagents and to seed the cells. Imaging will be performed with a Cellomics ArrayScan high-capacity HCS reader.
Results: We successfully differentiated stem cells into OPCs and RGCs and purified them. With our protocol, O4+ OPCs are detected as early as day 60 and myelin basic protein (MBP+) OLs within 100 days of differentiation. Using CRIPSR/Cas9, we have successfully generated a dual knock-In reporter cell line that expresses tdTomato driven by the PDGFRa promoter, which should express GFP when MBP is expressed. We also have a stem cell reporter line for RGCs that expresses tdTomato driven by the RGC specific gene POU4F2/BRN3B. In addition, using our reporter cell lines, we can purify PDGFRa+/tdTomato+ OPCs and tdTomato+/POU4F2+ RGCs with 90% and 95% purity, respectively.
Conclusions: With successful differentiation and purification of stem cell-derived RGC and OPC reporter cells, we are ready to initiate small molecule screening to identify compounds that promote myelination.
Commercial Relationships: xitiz chamling; **Valentin Sluch**, None; **Karl J. Wahlin**, None; **Donald J. Zack**, None

Program Number: 5904 **Poster Board Number:** B0496
Presentation Time: 11:30 AM–1:15 PM
Investigating temporal mechanisms of retinal interneuron loss and dysfunction caused by Atrx deficiency
Pamela S Lagali[1, 2], Brandon Y. Zhao[1], Adam Baker[1, 2], Stuart G. Coupland[2, 1], Keqin Yan[1], David J. Picketts[1], Catherine Tsilfidis[1, 2]. [1]Regenerative Medicine, Ottawa Hospital Research Institute, Ottawa, ON, Canada; [2]University of Ottawa Eye Institute, Ottawa, ON, Canada.

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to **http://iovs.arvojournals.org/** to access the versions of record.

Purpose: Deficiency of the chromatin remodeling protein Atrx in the mouse retina causes the selective loss of amacrine and horizontal cells and inner retinal dysfunction. Onset of the neurodegenerative phenotype coincides with both eye opening and developmental maturation of retinal interneurons and their circuitry. We aim to determine the contributions of these biological processes in causing the cellular and functional defects observed in Atrx-deficient mice. Here we interrogate the influence of light-dependent visual signaling on the survival of inhibitory interneurons in the mouse retina to determine the relevance of eye-opening on triggering the Atrx-knockout phenotype.
Methods: Retina-specific Atrx conditional knockout (cKO) mice were generated using Chx10-GFP/Cre-IRES-AP and Atrx-floxed lines. Photic input was blocked in the Atrx cKO mice using two separate paradigms: 1) genetic deficiency of metabotropic glutamate receptor 6 (Grm6) in *Chx10Cre*$^{+/-}$*;Atrx*$^{fl/y}$*;Grm6*^{nob4} triple transgenic mice, and 2) a dark-rearing regime. Retinal immunohistochemistry and enumeration of amacrine and horizontal cells were performed for both light deprivation paradigms. Scotopic electroretinography was performed for adult dark-reared Atrx cKO mice and controls to measure scotopic a-wave, b-wave, and oscillatory potential amplitudes.
Results: The Atrx cKO mice contained one-third fewer amacrine and horizontal cells compared to wildtype controls. The *Grm6*^{nob4} mice exhibited a 10-15% reduction in the retinal inhibitory interneuron population relative to wildtype mice. The number of retinal interneurons was further reduced in the Atrx/Grm6 double mutants compared to Atrx cKO mice. Deficits were observed in the scotopic b-wave and oscillatory potential amplitudes of dark-reared Atrx cKO mice similar to light-exposed counterparts.
Conclusions: The loss of retinal inhibitory interneurons in Atrx cKO mice is exacerbated by the blockage of light signaling through the ON pathway in Atrx/Grm6 double mutant mice. Visual deprivation by dark-rearing also does not rescue the functional deficiency caused by Atrx deletion. Our results indicate a protective effect of visual signaling on the retinal interneuron population and suggest that the temporal features of the Atrx cKO phenotype are not directly related to light exposure upon eye opening but rather coincident developmental processes impacting the inner retinal circuitry.
Commercial Relationships: Pamela S Lagali, None; **Brandon Y. Zhao**, None; **Adam Baker**, None; **Stuart G. Coupland**, None; **Keqin Yan**, None; **David J. Picketts**, None; **Catherine Tsilfidis**, None
Support: Foundation Fighting Blindness-Canada

Program Number: 5905 **Poster Board Number:** B0497
Presentation Time: 11:30 AM–1:15 PM
An Avian Adeno-Associated Viral Vector for Visualization of Post-Natal Chick Retinal Circuitry
Derek Waldner[1, 2]*, Frank Visser*[1, 2]*, William K. Stell*[3, 2]. [1]Cumming School of Medicine, University of Calgary, Calgary, AB, Canada; [2]Hotchkiss Brain Institute, Calgary, AB, Canada; [3]Cell Biology and Anatomy, University of Calgary, Calgary, AB, Canada.
Purpose: The post-hatching chicken is a valuable animal model for research, but the molecular tools needed for altering its gene expression are not yet available. Our purpose here was to adapt the adeno-associated viral (AAV) vector method, used widely in mammalian studies, for use in investigations of the chicken retina. We hypothesized that the recently characterized avian adeno-associated viral (A3V) vector could effectively transduce chick retinal neurons for *in situ* visualization, morphological characterization, and ultimately, genetic manipulation.
Methods: A3V encoding green fluorescent protein (A3V-GFP) was produced via the triple-plasmid transfection method and iodixanol gradient purification. Concentrated A3V-GFP (10^{11}-10^{12} genome copies/mL) was co-injected with varying concentrations of the glycosidic enzymes heparinase III (E.C. 4.2.2.8) and hyaluronan lyase (E.C. 4.2.2.1) into P1-3 chick vitreous humour and incubated for 7-10 days. Whole retinas were then flat-mounted and visualized via laser-scanning confocal microscopy for analysis of expression and imaging of individual retinal cells. ImageJ v2.0.0, Simple Neurite Tracer v3.0.5, and Volume Viewer v2.01 were used for imaging and reconstruction of retinal cells.
Results: Intravitreal A3V-GFP injection resulted in GFP expression in a small percent of retinal cells, primarily those with processes and/or cell bodies near the vitreal surface (ganglion cells, Müller cells). Co-injection of glycosidic enzymes increased the depth and breadth of viral transduction, allowing for visualization of more outer retinal neurons. Reconstruction of isolated cells allowed for computational analysis and 3D visualization of morphology.
Conclusions: Intravitreal A3V injection is a promising method for investigating chick retinal cells and circuitry *in situ*. The use of viral vectors uniquely allows for the use of cell-specific promoters, which may be used to unravel morphologies of specific marker expressing cell-types. Future developments to increase transduction efficiency of A3V may allow for its use in gene knock-in and knockout experiments, thus vastly increasing the experimental potential of this valuable animal model.



Retinal cells expressing GFP following transduction with A3V-GFP, including a retinal ganglion cell (left, compressed Z-stack), horizontal cell (middle, compressed Z-stack) and Müller glial cell (right, 3D reconstruction). Scale bars = 25 μm.
Commercial Relationships: Derek Waldner, None; **Frank Visser**, None; **William K. Stell**, None
Support: This work is supported by a NSERC Discovery Grant (WKS; RGPIN/131-2013), a Lions Sight Centre Fund Seed Grant, University of Calgary (WKS), a Foundation Fighting Blindness [Canada]-EYEGEYE Research Training Award (DW), and the Molecular Core Facility, Hotchkiss Brain Institute, Cumming School of Medicine (FV)

Program Number: 5906 **Poster Board Number:** B0498
Presentation Time: 11:30 AM–1:15 PM
Non-invasive, *in vivo* assessment of retinal cell death in light-induced retinal degeneration
Claudia Mueller, Francesca Mazzoni, Silvia Finnemann. Department of Biological Sciences, Fordham University, Bronx, NY.
Purpose: Detecting retinal cell death in animal models for eye disease using non-invasive, non-toxic live imaging will benefit translational vision research. We previously found that eye drops containing PSVue, a fluorescent probe that binds phosphatidylserine (PS) displayed by dying cells, label dying photoreceptors in the mutant Royal College of Surgeons (RCS) rat. As RCS rat RPE lacks the capability for PS-dependent clearance phagocytosis, excessive levels of PS-marked debris accumulate in degenerating RCS retina.

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to **http://iovs.arvojournals.org/** to access the versions of record.

Here, we ask if PSVue eye drops detect dying photoreceptors in induced retinal degeneration, in which wild-type RPE has normal capacity to remove PS-marked debris.
Methods: Retinal cell death was induced by exposing dark adapted albino rats to white light at 10,000 lux for 1 hour, followed by 6,000 lux for 23 hours. Control rats were dark adapted but not exposed to bright light. Retinal degeneration was followed for up to 6 days after light exposure by marker immunohistochemistry and immunoblotting. 5 days after light damage rats received PSVue or vehicle only as eye drop. Fluorescence imaging was performed 24 h later either *ex situ* on live dissected neural retina by microscopy or *in vivo* on live rats using a Kodak FX-Pro imager. All experiments were performed 3 times independently with 3-4 animals per group. Fluorescence levels were quantified, ratios of PSVue to control signals calculated and analyzed by Student's t-test.
Results: Changes in retinal morphology were obvious starting 5 days after light damage including rosette formation, outer segment thinning and reduction in photoreceptor nuclei. Immunoblotting confirmed ongoing retinal degeneration and partial photoreceptor loss. Tissue dissection revealed that PSVue applied as eye drops labeled dying photoreceptors as early as 3 days after and was robust in all rats 5 days after light exposure. *In vivo* live imaging detected significantly increased fluorescence in all rats 5 days after light damage compared to control rats.
Conclusions: Our results show that PS-probe applied as eye drops detects PS-marked cell debris in acutely induced photoreceptor degeneration in wild-type rats with intact phagocytic activity of the RPE. We suggest that PS-probe eye drops have utility as non-invasive, efficient screening method to monitor ongoing retinal degeneration in a wide range of animal models.
Commercial Relationships: Claudia Mueller, None; **Francesca Mazzoni**, None; **Silvia Finnemann**, None
Support: NIH grant R01-EY026215 and Beckman Initiative for Macular Research Award 13-06

Program Number: 5907 **Poster Board Number:** B0499
Presentation Time: 11:30 AM–1:15 PM
Preservation of retinal function by paeoniflorin following retinal light injury
Paul Park[1], Crystal Algenio[1], ZHIQUN TAN[2], James F. McDonnell[1], Liang Qiao[3], Jay I. Perlman[1, 4], Ping Bu[1, 5]. [1]Ophthalmology, Loyola University Medical Center, Maywood, IL; [2]Institute for Memory Impairments and Neurological Disorders, University of California Irvine, Irvine, CA; [3]Microbiology and Immunology, Loyola University Medical Center, Maywood, IL; [4]Surgery Service, Edward Hines, Jr. VA Hospital, Hines, IL; [5]Research Service, Edward Hines, Jr. VA Hospital, Hines, IL.
Purpose: Age-related macular degeneration (AMD) is a leading cause of blindness in the United States and other developed nations. Current treatment modalities targeting AMD are beneficial to only a small proportion of patients with the end-stage form and do not necessarily prevent progression of the disease. Paeoniflorin (PF) is one of the main active components of Paeonia Radix, a traditional Chinese herbal medicine derived from the root of Paeonia lactiflora Pallas. Paeoniflorin possesses both anti-inflammatory and anti-oxidant properties and has been implicated as a potential therapy in other degenerative pathologies such as Alzheimer's disease. We hypothesize that paeoniflorin will have protective effects against retinal degeneration in a mouse model of retinal light injury.
Methods: BALB/c albino mice were assigned to one of two groups: vehicle-treated (control) or paeoniflorin-treated retinal light injury mice. Vehicle (PBS) or paeoniflorin (20 mg/kg in PBS) was injected intraperitoneally, and mice were then exposed to 10,000 lux cool white fluorescent light for 2 hours to induce light injury. Both groups had treatment administered once daily for 4 additional days following light injury for a total of 5 treatments. Scotopic electroretinography (ERG) was recorded before light injury and 4 days following light injury.
Results: Before treatment, scotopic ERG a- and b-wave amplitudes were an average of 542 ± 31 μV and 1005 ± 61 μV, respectively, in the control group and 452 ± 109 μV and 867 ± 147 μV, respectively, in the paeoniflorin treatment group. Following retinal light injury, ERG a-wave amplitudes were 132 ± 17 μV in the control group and 250 ± 59 μV in the paeoniflorin treatment group ($p < 0.005$). Following retinal light injury, ERG b-wave amplitudes were 304 ± 42 μV in the control group and 518 ± 138 μV in the paeoniflorin treatment group ($p < 0.005$). There was significantly greater preservation of ERG a- and b-wave amplitudes in the paeoniflorin treatment group, suggesting that paeoniflorin is able to attenuate the degree of degenerative loss of retinal function in a retinal light injury model.
Conclusions: Paeoniflorin treatment of mice had a significant impact on the preservation of ERG a-and b-wave amplitudes following retinal light injury when compared to a control group. Our preliminary findings suggest that paeoniforin may have therapeutic value in the management of retinal degenerative conditions.
Commercial Relationships: Paul Park, None; **Crystal Algenio**, None; **ZHIQUN TAN**, None; **James F. McDonnell**, None; **Liang Qiao**, None; **Jay I. Perlman**, None; **Ping Bu**, None
Support: Illinois Society for the Prevention of Blindness, The Richard A. Perritt Charitable Foundation

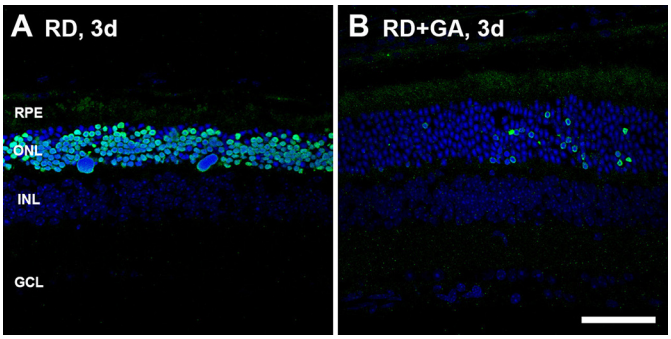
Program Number: 5908 **Poster Board Number:** B0500
Presentation Time: 11:30 AM–1:15 PM
Neuroprotective role of activated AMPK against light-induced photoreceptor cell death
Hirohiko Kawashima, Tomohiro Okamoto, Mamoru Kamoshita, Norihiro Nagai, Kazuo Tsubota, Yoko Ozawa. ophthalmology, Keio University Hospital, Shinnjuku-ku, Japan.
Purpose: The retina is known as a highly metabolic tissue. Using light-induced retinal degeneration model mice, we evaluated the neuroprotective effect of activated AMP-activated protein kinase (AMPK) which plays a crucial role in metabolism.
Methods: Seven- to 8-week-old BALB/c mice were divided into 4 groups and treated either with control vehicle or an AMPK activator, 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) at 125 or 250 or 500 mg/kg body weight, twice, before and after 12 hours of dark adaptation. The mice were then, exposed to a white fluorescent lamp for 1 hour at 3000 lux. The TUNEL positive and apoptotic cells were counted in the retina 2 days after light exposure. Full-field scotopic electroretinogram was recorded in response to the flash light stimuli at intensities ranging from –2.12 to 2.89 log cds/m^2, 4 days after the exposure.
Results: The TUNEL positive and apoptotic cells were only found in the photoreceptor layer. The increase in the number of apoptotic cells in the light exposed retina was suppressed by AICAR. The decrease in the amplitudes of a-waves 4 days after light exposure was attenuated by in the AICAR treated group in a dose dependent manner. Along with this, decreased in b-wave amplitudes were also attenuated in the AICAR treated group under some of the stimulus condition. There were no changes in the implicit times of a-wave and b-wave.
Conclusions: Both histological and functional analyses showed suppression of photoreceptor degeneration induced by light exposure in the AICAR treated group. Although further studies are required,

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to **http://iovs.arvojournals.org/** to access the versions of record.

AMPK activation may have a neuroprotective effect against light-induced photoreceptor degeneration.
Commercial Relationships: Hirohiko Kawashima, None; **Tomohiro Okamoto**, None; **Mamoru Kamoshita**, None; **Norihiro Nagai**, None; **Kazuo Tsubota**, None; **Yoko Ozawa**, None

Program Number: 5909 **Poster Board Number:** B0501
Presentation Time: 11:30 AM–1:15 PM
Low Dose Sodium Iodate Produces a Strain-dependent Retinal Oxidative Stress Response
Bruce A. Berkowitz, Jacob Lenning, Nikita Khetarpal, Catherine Tran, Johnny Wu, Robin Roberts. Anatomy/Cell Biol & Ophthal, Wayne State Univ Sch of Med, Detroit, MI.
Purpose: To compare biomarkers of pathogenic retinal oxidative stress, and its functional consequences, in two common mouse strains treated with sodium iodate.
Methods: C57BL6/J (B6) and 129S6 (S6) mice were treated with 20 mg/kg IP sodium iodate ± antioxidant methylene blue (MB) or vehicle, and then dark adapted. The next day, dark adapted layer-specific free radical production *in vivo*, and retinal thickness, were measured from 1/T1 MRI data (PMID: 26670830). Other sodium iodate treated groups were also studied the next day for retinal superoxide levels *ex vivo* (Lucigenin), contrast sensitivity (CS, optokinetic tracking) ± MB+α-lipoic acid (dosed per PMID: 26670830), and optical coherence tomography (OCT).
Results: Compared to vehicle injected mice, MB quenched ($p < 0.05$) supernormal 1/T1 in the outer nuclear layer (48-72% depth) of S6 mice, and in the retinal pigment epithelium/outer segment layer (88-100% depth) of B6 mice. In these responsive regions, outer retinal 1/T1 was 33% lower ($p < 0.05$) in treated S6 mice than in treated B6 mice; whole retinal superoxide production was 46% lower ($p < 0.05$) in treated S6 than in treated B6 mice. In treated B6 and S6 mice, CS was 24% and 16% subnormal, respectively; MB+α-lipoic acid treatment improved CS only in the B6 group. Retinal thicknesses (OCT, MRI) were normal in treated B6 and S6 mice.
Conclusions: Across biomarkers, low dose sodium iodate caused significantly less retinal oxidative stress in S6 than in B6 mice. Identifying strain-dependent oxidative stress responses will help in the study of the pathogenesis of retinal degeneration.
Commercial Relationships: Bruce A. Berkowitz, None; **Jacob Lenning**, None; **Nikita Khetarpal**, None; **Catherine Tran**, None; **Johnny Wu**, None; **Robin Roberts**, None
Support: NIH R21 EY021619; R01 EY026584; an unrestricted grant from Research to Prevent Blindness

Program Number: 5910 **Poster Board Number:** B0502
Presentation Time: 11:30 AM–1:15 PM
Glycyrrhizic acid attenuates retinal degeneration induced by blue light-emitting diode exposure in mice
In-Beom Kim[1, 2], Gyu Hyun Kim[1, 2], Sun-Sook Paik[1, 2], Yongsoo Park[1, 2], Hyung Il Kim[3]. [1]Department of Anatomy, Catholic University of Korea, Seoul, Korea (the Republic of); [2]Catholic Neuroscience Institute, Seoul, Korea (the Republic of); [3]Gyeongju St. Mary's Eye Clinic, Gyeongju, Korea (the Republic of).
Purpose: The root and rhizomes of licorice (*Glycyrrhiza*) have been used as an herbal medicine, because they have various therapeutic effects, such as anti-inflammatory and antioxidative activities. Glycyrrhizic acid (GA) is a major component in the root and rhizomes of licorice. In this study, we examined the effect of GA in an animal model for retinal degeneration (RD), which is the leading cause of blindness and characterized by the irreversible and progressive degeneration of photoreceptor cells in the retina.
Methods: RD was induced in BALB/c mice by exposure to a blue light-emitting diode (LED) (460 nm) for 2 hours. To examine retinal functions, electroretinography (ERG) was performed. To assess histopathological changes, hematoxylin and eosin (H&E) staining were conducted. Apoptotic cell death was evaluated by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. In addition, changes in proinflammatory cytokines were detected by real time RT-PCR and retinal stress and inflammation were evaluated by immunohistochemistry with anti-ionized calcium binding adaptor molecule 1 (Iba-1) and anti-glial fibrillary acidic protein (GFAP).
Results: Scotopic ERG showed that both a- and b-waves were significantly reduced in RD mice, while amplitudes of both waves were significantly increased in GA-treated RD mice, compared to those in non-treated RD animals. H&E and TUNEL assay showed that the outer nuclear layer where photoreceptors reside appeared to be more preserved and less apoptotic cells were observed in GA-treated RD retinas than in non-treated RD retinas. GA reduced expression of proinflammatory cytokines, such as TNF-α, IL-6, IL-1β, CCL2 and 6, iNOS, and Cox-2. In addition, GA reduced expression of Iba-1 and GFAP, indicating decreased glial response, retinal stress and inflammation.
Conclusions: These results demonstrate that GA reduces retinal inflammation and prevents photoreceptor cell death from experimentally induced RD, suggesting that GA may have a potential for the treatment of RD as a medication.



Evaluation of protective effect of GA against photoreceptor cell death at 3 day after blue LED exposure by TUNEL staining
Commercial Relationships: In-Beom Kim, None; **Gyu Hyun Kim**, None; **Sun-Sook Paik**, None; **Yongsoo Park**, None; **Hyung Il Kim**, None
Support: Korea Health Industry Development Institute (KHIDI) Grant HI15C3076; Seoul St. Mary's Hospital Grant 2016

Program Number: 5911 **Poster Board Number:** B0503
Presentation Time: 11:30 AM–1:15 PM
Effects of bFGF on rescue of hydrochloroquine-induced damage of cultured human RPE cells
Dan-Ning Hu, Xilun Shen, Richard B. Rosen. New York Eye and Ear Infirmary of Mount Sinai, New York, NY.
Purpose: Long-term usage of hydroxychloroquine (HCQ) for the treatment of autoimmune diseases often leads to HCQ retinopathy. This is caused mainly by the inhibition of lysosomal enzyme activity, which leads to the blockage of autophagy, resulting in an increase in vacuolation and cell death. We established an *in vitro* model using cultured human RPE cells to evaluate the effects of various factors on the protection of RPE cells against HCQ toxicity and tested the effects of basic fibroblast growth factor (bFGF) on this process.
Methods: ARPE 19 cell line and a primary cell culture of human RPE cells were seeded into 12-well and 96-well plates and treated

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to **http://iovs.arvojournals.org/** to access the versions of record.

with HCQ at 10 -100 μM with or without bFGF (10 ng/ml) for 3 days. Morphological changes and cell viability were evaluated using phase-contrast microscopy and MTT studies. All experiments were performed in triplicate.
Results: RPE cells cultured with 30 μM HCQ showed a marked increase of vacuolation in the cytoplasm, without changes of cell viability. Cells cultured with 100 μM HCQ showed a significant decrease in cell viability to 16%±2% of the control ($P<0.05$). bFGF did not affect the accumulation of vacuolation in cells treated with 30 μM HCQ, but significantly increased the viability of cells treated with 100 μM HCQ alone (4.3 fold) ($P<0.05$). However, the number of viable cells was still less than cells not treated with HCQ (69 ±8% of the control, $P<0.05$), suggesting that bFGF has a significant but only partial protective effect on HCQ-induced cell death.
Conclusions: HCQ-induced RPE cells damage can be evaluated using this *in vitro* model. bFGF partially improves cell viability of RPE cells treated by HCQ, but does not reduce the accumulation of vacuolation. This suggests that bFGF cannot restore the function of the lysosomes and that the decrease of cell death is possibly a non-specific cell survival effect. Further exploration of potential agents which can recover lysosomal enzyme activity and restore autophagy will be necessary in order to develop a novel therapy for HCQ retinopathy.
Commercial Relationships: Dan-Ning Hu, None; **Xilun Shen**, None; **Richard B. Rosen**, None
Support: Supported by the Marrus Family Foundation and the New York Eye and Ear Foundation

Program Number: 5912 **Poster Board Number:** B0504
Presentation Time: 11:30 AM–1:15 PM
Selective RPE-ablation of dehydrodolichyl diphosphate synthase (DHDDS) causes morphologic changes similar to geographic atrophy in dry AMD
Steven J. Pittler[1], Stephanie Davis[1], Sriganesh Ramachandra Rao[2, 3], Delores A. Stacks[1], Marci L. DeRamus[1], Steven J. Fliesler[4, 3]. [1]Optometry and Vision Science, University of Alabama at Birmingham, Birmingham, AL; [2]Biochemistry, SUNY-University at Buffalo, Buffalo, NY; [3]Research Service, VA Western NY Healthcare System, Buffalo, NY; [4]Ophthalmology, Biochemistry, & Neuroscience Program, SUNY-University at Buffalo, Buffalo, NY.
Purpose: DHDDS (RP59) is required for the synthesis of functional dolichols that are essential for N-linked glycosylation of proteins. To assess the effects of selective ablation of DHDDS in RPE we generated and analyzed *Dhdds* conditional knockout mice.
Methods: A Cre conditional knockout construct (KOMP, UC Davis) was bred onto a C57Bl/6J background, and these mice were crossed to VMD2-RPE-Cre mice (Yun Le, OUHSC). The resulting homozygous (hz) and heterozygous (het) RPE-*Dhdds*-ko, VMD2-RPE-Cre, and C57Bl/6J control lines were analyzed by fundus exam, fluorescein angiography, histology, ERG, and optokinetic reflex (OKR). OKR was performed at a constant speed of 12d/s and contrast sensitivity determined at several spatial frequencies. Student's t-test was used to compare differences between group responses (stat. signif., $p<0.05$).
Results: No pathology was observed in retinas of het RPE-*Dhdds*-ko or control lines examined from 1 to 3 mo. Abnormal pigmentation was observed in the fundus of 8- and 10-mo old hz RPE-*Dhdds*-ko mice; vascular changes, including microanuerisms, increased vessel tortuosity and attenuation, were also observed by 6 mo of age. Homozygous RPE-*Dhdds*-ko mice at 2 and 3 mo displayed geographic RPE atrophy, photoreceptor shortening and loss, RPE transmigration into the retina, and external limiting membrane (ELM) descent towards Bruch's membrane. At 2 mo of age, hz and het RPE-*Dhdds*-ko mice exhibited reduced scotopic ERG a- (39% and 17%) and b-wave (49% and 40%) responses compared to WT ($p<0.05$). At 3 mo, scotopic ERG a-wave amplitudes were reduced in hz RPE-*Dhdds*-ko (83%), while b-wave responses were reduced in both genotypes (84% and 28%, $p<0.05$). Photopic ERG responses in hz RPE-*Dhdds*-ko mice showed reductions in a- (86%) and b-wave (95%) responses ($p<0.05$). OKR analysis showed reductions in photopic (4-31%) and scotopic (8-29%) contrast sensitivity over the range of 0.031 to 0.272 c/d in 3-mo old hz RPE-*Dhdds*-ko mice ($p<0.05$). No differences in spatial acuity were observed.
Conclusions: Potential perturbation of DHDDS-dependent processes selectively in the RPE may cause the observed structural and functional deficits in RPE and photoreceptors. The slowly progressing nature of these defects suggests there may be a window of opportunity to identify key proteins that are involved in directing the observed dry AMD-like pathology.
Commercial Relationships: Steven J. Pittler, None; **Stephanie Davis**, None; **Sriganesh Ramachandra Rao**, None; **Delores A. Stacks**, None; **Marci L. DeRamus**, None; **Steven J. Fliesler**, None
Support: Facilities and resources of UAB School of Optometry, UAB Vision Science Research Center, P30 EY003039, R01 EY018143 (SJP); R01EY007361 (SJF); RPB Unrestricted Grant (SJF); Facilities and resources of the VAWNYHS (SJF).

Program Number: 5913 **Poster Board Number:** B0505
Presentation Time: 11:30 AM–1:15 PM
Therapeutic Effects of Paeoniflorin Following Retinal Ischemic-Reperfusion Injury
Crystal S. Algenio[1, 2], Mark Qiao[1], Sabrina Chow[3], James F. McDonnell[1], Jay Perlman[5, 4], Ping Bu[1, 2]. [1]Ophthalmology, Loyola University Medical Center, Maywood, IL; [2]Research Services, Edward Hines, Jr. VA Hospital, Hines, IL; [3]Microbiology and Immunology, Loyola University Medical Center, Maywood, IL; [4]Surgery Service, Edward Hines, Jr. VA Hospital, Hines, IL; [5]Ophthalmology/Pathology, Loyola University Medical Center, Maywood, IL.
Purpose: Retinal ischemia-reperfusion presents a major common cause of visual impairment and blindness in the industrialized world. Current treatment options for retinal ischemic diseases are very limited. Paeoniflorin is a monoterpene glycoside derived from the root of *Paeonia lactiflora* Pallas. Paeoniflorin has been shown in previous studies to exhibit various pharmacological properties including anti-inflammatory, antioxidant, and immunoregulatory activities, making it a potential therapeutic agent against degenerative pathologies. The purpose of this study is to determine if Paeoniflorin attenuates retinal degeneration by minimizing oxidative and inflammatory stress in retinal ischemic-reperfusion injury.
Methods: C57BL/6 mice were assigned to one of two groups: vehicle- treated or Paeoniflorin-treated retinal ischemic injury mice. Retinal ischemia was induced by transient elevation of intraocular pressure for 45 minutes. Vehicle (PBS) or Paeoniflorin (20 mg/kg in PBS) was injected intraperitoneally once a day for 5 days following retinal ischemia. Scotopic electroretinography (ERG) was recorded before induction of retinal ischemia, and 7 days after inducing ischemia. The amplitude and times of the scotopic a- and b-waves were quantified and statistically compared among Paeoniflorin treatment and control groups.
Results: Before treatment, scotopic ERG a- and b-wave amplitudes were 392 ± 75 μV and 831 ± 62 μV, respectively, in the control group, and 396 ± 43 μV and 818 ± 75 μV, respectively, in the Paeoniflorin group. Following ischemic-reperfusion injury, ERG a-wave amplitudes were 180 ± 13 μV in the control group and 307

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to **http://iovs.arvojournals.org/** to access the versions of record.

± 61 μV in the Paeoniflorin group ($p < 0.005$). Post-ischemic insult ERG b- wave amplitudes were 320 ± 48 μV in the control group and 583 ± 74 μV in the Paeoniflorin group ($p < 0.005$). Seven days after ischemic-reperfusion injury, Paeoniflorin treatment of mice was proven to attenuate ischemic induced loss of retinal function compared to vehicle treated mice, as shown by the preservation of ERG a- and b-wave amplitudes.
Conclusions: Conclusions: Paeoniflorin treatment of mice showed attenuation of retinal degeneration by preservation of ERG a-and b-wave amplitudes following ischemic-reperfusion injury. Our preliminary data suggests that Paeoniflorin may have therapeutic value in the management of retinal ischemic diseases.
Commercial Relationships: Crystal S. Algenio, None; **Mark Qiao**, None; **Sabrina Chow**, None; **James F. McDonnell**, None; **Jay Perlman**, None; **Ping Bu**, None
Support: Illinois Society for the Prevention of Blindness, The Richard A. Perritt Charitable Foundation

Program Number: 5914 **Poster Board Number:** B0506
Presentation Time: 11:30 AM–1:15 PM
The effect of MIF inhibitor ISO-1 on apoptosis in chick retinal damage model
Rania Kusibati[1], Bongsu Kim[1], Tyler Heisler-Taylor[1], Levi Todd[2], Andy J. Fischer[2], Colleen M. Cebulla[1]. [1]Havener Eye Institute, Ophthalmology and Visual Science, The Ohio State University, Columbus, OH; [2]Neuroscience, The Ohio state University, Columbus, OH.
Purpose: Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine which may play a role in neuronal apoptosis after retinal damage. In this study we evaluated the retinal expression and localization of MIF after excitotoxic retinal injury in the chick. We also tested if the intraocular injections of the MIF inhibitor ISO-1 will affect retinal apoptosis.
Methods: Under an IACUC-approved protocol P7 Leghorn chicks (Gallus gallus domesticus) were treated with intravitreal injection of NMDA 500 nmol or vehicle and evaluated for MIF expression with immunohistochemistry (IHC) (n=6). Apoptosis was detected with TUNEL assay. A dose-finding study (n= 4-6/group) was conducted in order to determine the maximum effective dose of ISO-1 which would significantly decrease NMDA-induced apoptosis. Chicks received a pre-treating dose of vehicle or ISO-1 (250 μmol) 24 hours before intraocular NMDA injection and a second dose of different doses of ISO-1 (25 μmol - 20 mmol) on day 0. Eyes were enucleated; retinal TUNEL was evaluated on day 1 and images were analyzed via Image J. Paired t-test was used for statistical analysis.
Results: IHC demonstrated a markedly increased elevation of MIF expression in the cytoplasm of inner retinal neurons after NMDA damage compared to non-damaged control eyes. The dose finding study showed that the intravitreal injection of 10 mmol ISO-1 produced the most statistically significant decrease in TUNEL positive cells in the inner nuclear layer (INL) 24 hours after NMDA injection (9756.4 ± 1646.7 cells/mm^2 retina vehicle vs 4566.3 ± 1443.8 cells/mm^2 retina ISO-1, $p=0.02$).
Conclusions: The neuronal upregulation of MIF in the damaged chick retina suggests this model can be a valid tool for evaluating how MIF impacts neuronal survival after excitotoxic retinal injury. The significant reduction in apoptosis by intravitreal injection of ISO-1 at day 1, suggests ISO-1 as a potential neuroprotective agent against retinal damage.
Commercial Relationships: Rania Kusibati, None; **Bongsu Kim**, None; **Tyler Heisler-Taylor**, None; **Levi Todd**, None; **Andy J. Fischer**; **Colleen M. Cebulla**, None
Support: NIH K08EY022672, P30 CA016058 (OSU-CCC Nucleic Acids Shared Resource), NCATS KL2TR001068. EY014801 (core grant of BPEI). The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding institutions. Additional funds were provided by the Ohio Lions Eye Research Foundation, Ophthalmology Fund #313310, and a grant (EY022030-3) from the National Eye Institute, National Institutes of Health.

Program Number: 5915 **Poster Board Number:** B0507
Presentation Time: 11:30 AM–1:15 PM
Regeneration of the retinal pigment epithelium in a novel zebrafish model of macular degeneration
Nicholas Hanovice[1, 2], Ross F. Collery[3], Brian A. Link[3], Jeffrey M. Gross[2]. [1]Molecular Biosciences, University of Texas at Austin, Pittsburgh, PA; [2]Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA; [3]Cell Biology, Neurobiology & Anatomy, Medical College of Wisconsin, Milwaukee, WI.
Purpose: Geographic Age-Related Macular Degeneration results from progressive atrophy of the retinal pigment epithelium (RPE), which leads to photoreceptor (PR) degeneration and blindness. While much has been learned about stem cell-based RPE transplant therapies, our understanding of RPE regeneration is incomplete. Zebrafish display a robust regenerative response to injury; thus we hypothesized that the RPE would successfully regenerate. Furthermore, in mammals, proliferation of neighboring RPE cells has been associated with limited endogenous repair. We therefore hypothesized that unaffected RPE proliferates to repair RPE injury.
Methods: We utilized a novel zebrafish transgenic that enables nitroreductase-mediated ablation of central RPE cells in larval zebrafish. To characterize the dynamics of cell death following the ablation of RPE, we performed immunohistochemistry to detect TUNEL-positive nuclei. To characterize the recovery of RPE cells, we performed BrdU incorporation assays and immunohistochemistry using markers of RPE and PR cells at varying time points following ablation.
Results: Ablation of the RPE leads to apoptosis and degeneration of cells within the PR layer as early as 6 hours post injury, and the outer nuclear layer is significantly depopulated by 5 days post injury (dpi). By 7dpi, the PR layer becomes more organized, although limited RPE recovery is detectible. By 14dpi, a morphologically normal RPE and PR layer is detectable in the eye periphery. By 21dpi, regenerated RPE is detectible throughout the eye and the PR layer is completely recovered. BrdU incorporation assays reveal the presence of proliferative cells in the RPE layer following ablation. Proliferative cells are concentrated in the periphery soon after ablation and but appear more centrally as regeneration proceeds, mirroring the pattern of regenerating RPE cells.
Conclusions: These results establish a novel zebrafish paradigm through which the RPE can be specifically ablated, and are the first to demonstrate that the zebrafish RPE has the capacity to regenerate. Our data also suggest that proliferation of uninjured RPE cells in the periphery contributes to the regenerative response, and this is being tested by genetic lineage tracing analyses. This model enables the study of the molecular and cellular mechanisms underlying the regenerative response of the RPE in zebrafish.
Commercial Relationships: Nicholas Hanovice, None; **Ross F. Collery**, None; **Brian A. Link**, None; **Jeffrey M. Gross**, None
Support: Bright Focus M2016067

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to **http://iovs.arvojournals.org/** to access the versions of record.

Program Number: 5916 **Poster Board Number:** B0508
Presentation Time: 11:30 AM–1:15 PM

Lutein and zeaxanthin isomers protect photoreceptors against blue light-induced degeneration

Minzhong Yu[1, 2], Craig Beight[1, 3]. [1]Cole Eye Institute, Cleveland Clinic Foundation, Cleveland, OH; [2]Ophthalmology, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, OH; [3]Louis Stokes Cleveland Veterans Affairs Medical Center, Cleveland, OH.

Purpose: Oxidative stress and endoplasmic reticulum stress are major factors underlying photoreceptor degeneration. Lutein and zeaxanthin isomers (L/Zi), consisting of lutein, zeaxanthin and mesozeaxanthin carotenoids, are found widely in many foods and protect against cell damage by ameliorating oxidative stress in retina. In this study, we examined the effect of L/Zi supplementation in a mouse model of light-induced photoreceptor degeneration.

Methods: L/Zi (10 mg/kg) dissolved in sunflower oil (SFO, 1 mg/ml) or equal volume of SFO as vehicle was administered by daily oral gavage to 2-month old BALBc/J mice in treatment group (n=7) and vehicle group (n=7), respectively for a 5 day period. On Day 2, animals were exposed for 1 hour to blue light (5,000 lux) obtained by filtering white fluorescent light by a filter which transmitted light between 380 and 570 nm (Midnight Blue 5940, Solar Graphics, Clearwater, FL). ERG was tested to show the functional change of retina. Nrf2 and GRP78 were tested by western blot.

Results: ERG amplitudes were significantly reduced in the vehicle control mice after light exposure as compared to the baseline collected before the light exposure. ERG amplitudes after light exposure were significantly higher in the L/Zi treated group than in the vehicle control group. These ERG amplitudes were lower than baseline, indicating incomplete protection. In light-exposed retinas, Nrf2 was upregulated and GRP78 was downregulated by L/Zi treatment.

Conclusions: Treatment with L/Zi can protect photoreceptors against degeneration induced by high intensity blue light. This treatment effect is probably related to the decrease of oxidative stress and endoplasmic reticulum stress by the L/Zi treatment. Future studies will examine the potential for L/Zi in inherited forms of photoreceptor degeneration involving oxidative stress.

Commercial Relationships: Minzhong Yu; **Craig Beight**, None

Support: Omni Active Health Technologies Ltd., India Research Grant, NIH P30EY025585 and Research to Prevent Blindness

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to **http://iovs.arvojournals.org/** to access the versions of record.