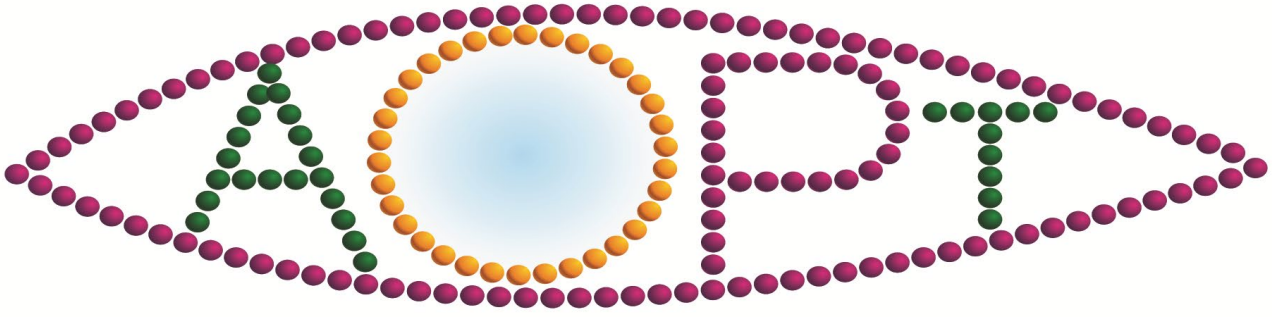
Poster Abstracts



Poster #1 Trabecular Meshwork Swelling and Osmoregulation

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Purpose: Trabecular meshwork (TM) plays a pivotal role in maintenance of intraocular pressure (IOP) within physiological ranges. Increase in TM resistance to aqueous humor outflow results in IOP elevation, optic neuropathy and vision loss in hypertensive glaucoma. TM outflow resistance is regulated dynamically but mechanisms are unclear. We hypothesize that mechanosensitive and swelling sensitive ion channels in transient receptor potential vanilloid (TRPV) and melastatin (TRPM) channel families interact with Na-K ATPases and aquaporins to regulate dose-dependent swelling of TM cells.

Methods: Immunostaining, calcium, sodium, potassium imaging, cell volume assays, whole cell patch-clamp electrophysiology and shRNA cell transfections demonstrated functional expression of TRPV, TRPM and Na-K ATPase family isoforms in primary and immortalized TM cells. Hypotonic stimulation and/or pharmacological agonists/antagonists were used to quantify the impact of mechanotransduction channels and pumps on the swelling response.

Results: Transmembrane current amplitudes and calcium signals showed dose-dependent swelling responses. Hypotonic stimulation induced structural changes by decreasing F-actin filament expression intensity. Intracellular calcium increases due to hypotonic stimulation required TRPV4 and TRPM4 activation for optimal responses. Hypotonic stimulation induced currents composed of intracellular calcium spikes and slower sodium currents associated with regulatory volume decrease. Cell current/voltage signals and calcium/sodium responses to hypotonic stimulation were attenuated varyingly by specific TRP channel antagonists.

Conclusions: These results identify TRPV4/TRPM4 interaction as a mechanism for TM osmosensing and subsequent cell swelling responses. Calcium signaling pathways, ideal ionic gradients for swelling through aquaporins, and whole TM tissue constriction/relaxation are likely mediated by these channels for regulating aqueous humor outflow.

Poster #2 Evaluating the safety of pharmaceutical drugs in a dual model of cell viability and barrier integrity

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Purpose: The aim of this work is to evaluate the safety of pharmaceutical drugs currently applied at a clinical level.

Methods: An in vitro corneal epithelial model was generated from human normal limbal cells grown on 1.12 cm^2 Transwell inserts and cultured for 7 days under air-lift conditions. Corneal toxicity and barrier disruptions were studied upon application of selected pharmaceutical drugs. Corneal toxicity was assessed via MTT assay while corneal barrier disruptions were assessed via variations in Trans-epithelial electrical resistance (TEER) before and after 30 minutes drug exposure and 120 minutes post-incubation in culture media. Toxicity and barrier disruptions were classified by a reduction in cell viability or barrier integrity in 40% or more.

Results: None of the evaluated ophthalmic drugs decreased cell viability in more than 10% overall, therefore, all formulations were classified as non-toxic. However, barrier integrity was highly affected upon application of several compounds, with decrements of almost 60% in initial TEER values.

Conclusions: The use of complementary test to cell viability via MTT could stablish new criteria for classification of current pharmaceutical products, relying not only on cell viability but in the barrier disruption potential. This test could be useful in distinguishing time-dependent and reversible damage to the ocular surface that is currently undervalued in current protocols for pharmacological safety.

Poster #3 ET-1 Treatment Reduces Expression of ATP5H and Cox17 in Retinal Ganglion Cells

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Purpose: Endothelin-1 (ET-1) treatment has been shown to promote apoptosis of retinal ganglion cells (RGCs), however, the precise mechanisms underlying these effects are still unknown. The purpose of the study was to assess the changes in gene expression at the level of translatome, occurring during endothelin-mediated neurodegeneration of RGCs.

Methods: Primary RGCs isolated from post-natal day 5 rat pups were treated with ET-1 (100 nM) for 24 h in trophic factor-free medium. Polysomal RNA was isolated and libraries for RNA-Seq were prepared. Trimmed mean of M-values (TMM) was used to normalize the gene expression. Genes with expression changes more than 1.5 fold with $p < 0.05$ were considered differentially expressed. Rats were intravitreally injected in one eye with 2 nmole of ET-1 and retina sections obtained were analysed for expression of ATP5H and Cox17.

Results: The STRING network analysis revealed mitochondrially relevant genes and out of the 156 differentially expressed genes, 23 genes were identified with known or predicted mitochondrial function. An increase in the expression of key mitochondrial genes including cytochrome c oxidase copper chaperone (Cox17) and ATP synthase, H^+ transporting mitochondrial F0 complex (ATP5H) was observed. However, a decrease in expression of ATP5H and Cox17 was found both in cultured RGCs treated with ET-1 as well as in retinal sections (primarily in the RGC layer) from rats eyes injected with ET-1.

Conclusions: ET-1 treatment produced changes in expression of key regulators of mitochondrial bioenergetics and oxidative metabolism which could be indicative of their involvement in neurodegeneration in glaucoma.

Poster #4 CX43 knockout human iPSC fail to generate normal retinal cups

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Purpose: Connexin43 (CX43) is a major protein that forms gap junction channels in embryonic stem cells. Mutations in the gene *Gap Junction Protein Alpha 1* (*GJA1*) expressing CX43 were identified in oculodentodigital dysplasia (ODDD) syndrome families. Symptoms include eye abnormalities such as microphthalmos. We established a model of ODDD using human iPSC-derived retinal cups. These may be useful to investigate if microphthalmos can be rescued by small molecules or gene therapy.

Methods: *GJA1* was disrupted using CRISPR/Cas9. The established lines were characterized by immunofluorescence and qPCR. The *GJA1* knockout cell lines and wild-type iPSC were differentiated into retinal organoids. Expression levels of neural makers and retinal markers were identified by qPCR and immunostaining.

Results: Using CRISPR/Cas9 genome editing, we successfully obtained multiple iPSC *GJA1* knockout clones. *GJA1*$^{-/-}$ iPSC remained undifferentiated and morphologically indistinguishable from wild-type iPSC. They remained typical iPSC morphology and had high expression level of pluripotency markers such as *OCT4*, *SOX2* and *NANOG*. *GJA1*$^{-/-}$ iPSCs had no defects in self-renewal and pluripotency state in primed states. However, after 26 days(d) differentiation, *GJA1*$^{-/-}$ iPSC failed to generate thick neuroepithelium in retinal organoids culture, which resulted in smaller retinal cups and thin neural retina. At d26 the neural identity marker *PAX6* was significantly lower than in the wild-type. Increased apoptotic cell death in *GJA1*$^{-/-}$ retinal cups were found at d50 as determined by immunoreactivity of CASPASE 3.

Conclusions: *GJA1* is not required for pluripotency in iPSC in primed states, but is required for iPSC to develop into normal retinal cups.

Poster #5 Therapeutic targeting of fibrosis and inflammation by novel aryl hydrocarbon receptor (AhR) ligands in neovascular age-related macular degeneration

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Purpose: AhR is a transcriptional regulator with pleiotropic functions in xenobiotic and lipid metabolism, vascular development, and cancer. Previously, we reported that loss of *AhR in vivo* exacerbates the severity of laser-induced choroidal neovascular (CNV) lesions. This led us to test the effect of AhR activation on angiogenesis and fibrosis, pathways associated with development and progression of CNV.

Methods: We screened AhR-active ligands (n=12) for their ability to activate AhR in choroidal endothelial cells (chEC), assessed their effect in *in vitro* functional angiogenesis assays and the *in vivo* laser-induced CNV model (C57BL/6J, 10-12 months, n=10 per group). Circulating cytokine (n=62) levels were measured by Mouse Cytokine ArrayC3. Based on the results from *in vitro* and *in vivo* studies, we designed and synthesized novel AhR ligands (n=5) and tested their activity, solubility, and toxicity *in vitro* and *in vivo*.

Results: We found that AhR-active ligands induced AhR promoter activity (5-12 fold), expression of AhR target genes (*CYP1A2*, *CYP1B1*), and inhibited VEGF-induced endothelial migration and tube-formation. Daily intraperitoneal treatment with AhR-active ligands alleviated the severity of laser-induced lesions (55-57%), compared to vehicle. AhR activation resulted in a decrease (33-37%) in collagen type IV accumulation in the CNV lesions, and reduction in the circulating levels of proinflammatory cytokines (n=8; $p < 0.01$).

Conclusions: Collectively, these findings validate the role of the AhR pathway in regulating the pathogenesis of CNV lesion formation and support our hypothesis that pharmacologic targeting of AhR may be used as potential therapy for the treatment of neovascular AMD.

Poster #6 Protecting Photoreceptor Function in Retinitis Pigmentosa with the Sphingolipid Analogue, FTY720

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Purpose: Retinitis Pigmentosa (RP) is an incurable and untreatable group of heterogeneous retinal neurodegenerative (RD) diseases that cause progressive dysfunction and death of retinal photoreceptor cells. A critical barrier for developing a therapy for RP is its heterogeneous nature, as it involves many pathways and genes. We've identified retinal ceramide, the signaling sphingolipid that is a potent mediator of cell death, increases in the retina and facilitates the retinal cell death process in many heterogeneous forms of degenerations. We hypothesized, regardless of the causal factor/gene, ceramide could be targeted to prevent and/or delay photoreceptor cell death. The purpose of this study was to test a Ceramide metabolic inhibitor for protection from retinal degeneration in mouse models of RP.

Methods: RD10 mice were given intraperitoneal injections with 2.5 mg/kg of FTY720 or Vehicle from 10 days (P10) and continued until the end of the experiment P60. Functional vision was measured by Optokinetic Tracking (OKT) at P30, P45, and P60. Likewise, photoreceptor function was measured by Electroretinogram (ERG) and retinal structure was measured in histological assays at P30, P45, and P60.

Results: Systemic delivery of ceramide inhibitor, FTY720, protected functional vision (OKT) in RD10 mice significantly by delaying the death of photoreceptor cells as observed in ERG and histological analyses.

Poster #7 TRPM8 Antagonist AMTB Impairs the Ability of Cold Thermosensitive Trigeminal Neurons Innervating the Ocular Surface to Encode Cold Stimulus Intensity

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Purpose: In the present work, we explored the effects of TRPM8 antagonist N-(3-aminopropyl)-2-{[(3-methylphenyl) methyl]oxy}-N-(2-thienylmethyl) benzamide hydrochloride salt (AMTB) on the background and stimulus-evoked activity of TG low threshold mechanosensory neurons (LTM), cold thermoreceptor, and mechano- and polymodal nociceptor neurons innervating the ocular surface (OS) of the anesthetized adult rat.

Methods: Impulse activity of TG neurons was recorded extracellularly with tungsten electrodes (1-2MΩ) and stored for off-line analysis with dedicated software. A microprobe thermometer was placed on the corneal surface for simultaneous temperature recording. Background impulse activity (BA) and the impulse response to cold (-0.1 to -20ºC), heat (+0.1 to +20ºC), chemical (hyperosmolar solutions, CO_2) and mechanical stimulation were analyzed in the different types of TG neurons innervating the OS. Background and stimulus-evoked activity before and 15 min after topical treatment with 1mM AMTB were compared.

Results: BA of cold thermoreceptor neurons was significantly reduced after ATMB (from 5.16±0.71 to 3.04±0.75 imp/s; $p<0.05$, n=15). Their response to cold stimulation was abolished by AMTB in 58.7% of recorded neurons, although when present the response to cooling stimuli did not encode the intensity of the cold stimulus. AMTB did not affect mechanical responsiveness of LTM neurons (n=6). Spontaneous and stimulus-evoked activity of mechano- and polymodal nociceptor neurons (n=5) was not significantly affected by AMTB.

Conclusions: Results confirms that cold thermosensitive TG neurons innervating the cornea and conjunctiva encode the intensity of temperature changes occurring on the ocular surface. This ability to encode cooling stimulation is mediated mainly by TRPM8, being abolished by AMTB.

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Poster #8 L-serine administration protects against neurovascular dysfunction in a mouse model of retinopathy of prematurity

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Purpose: Retinopathy of prematurity (ROP) is a vision-threatening neurovascular disorder and the leading cause of blindness in children worldwide. Emerging evidence indicate that neuronal energy demands drive the vascular development, particularly in photoreceptors which have the highest density of mitochondria in the body. Our knowledge about photoreceptor energy fuel requirement is very limited, particularly with respect to L-serine, although it has been shown to be critical to other highly metabolically active cancer cells. We aim to investigate the impact of L-serine on retinal neurovascular function in ROP.

Methods: In a mouse model of oxygen-induced retinopathy (OIR), we examined the retinal neuronal activity with L-serine or vehicle administration by electroretinography, photoreceptor structure with immunohistochemistry, and retinal vascular pathology with isolectin staining. Retinal use of L-serine as energy fuel was tested by Seahorse analysis. We also examined L- serine levels with ELISA, and retinal endogenous serine synthetic enzymes with Single-cell transcriptomics.

Results: In OIR, L-serine protected against cone dysfunction and normalized cone structure. L- serine also inhibits retinal neovascularization and this inhibition was abolished in photoreceptor- degenerating mice. L-serine increased mitochondrial oxygen consumption in isolated retinal punches *ex vivo* and photoreceptor cells *in vitro*. Circulating L-serine levels were induced in OIR versus normal mice and retinal serine synthetic enzymes were mostly expressed in Müller cells by Single-cell transcriptomics. Serine synthetic enzymes were highly induced in primary Müller cells isolated from OIR versus normal mouse retinas.

Conclusions: Our findings suggest that Müller-cell-derived L-serine may be energy fuel for photoreceptor metabolism and decrease neurovascular changes in ROP.

Poster #9 Loxl1 knockdown in primary optic nerve head astrocytes results in molecular and cellular phenotypes associated with reactive astrocytosis and elastinopathy

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Purpose: Exfoliation glaucoma is a common type of secondary open-angle glaucoma linked to single nucleotide polymorphisms in the lysyl oxidase-like 1 (*LOXL1*) gene. Non-coding variants in the *LOXL1* gene can result in reduced expression levels of Loxl1 protein in multiple ocular tissues, including the optic nerve head. The purpose of this study was to determine the effect of *Loxl1* down-regulation on optic nerve head astrocytes (ONHA).

Methods: Loxl1 knockdown was achieved by transfection of rat *Loxl1* gene-specific siRNA. For some experiments, a reactive astrocytic phenotype (reactive astrocytosis) was induced by exposure of ONHAs to either a humidified hyperbaric (20 - 25 mm Hg) atmosphere or to 10% static stretch. Quantitative PCR, immunoblotting and immunocytochemistry were performed using previously validated primers and antibodies.

Results: *Loxl1* expression was reduced by ~55% following siRNA transfection. Expression levels of GFAP (n = 5, $P < 0.001$) and voltage-gated calcium channel subunits (n = 3-4; $Ca_V\alpha$, $P < 0.01$; $Ca_V2.1$, $P < 0.01$) were significantly increased following *Loxl1* knockdown, suggestive of reactive astrocytosis. Concurrently, expression levels of elastin and collagen VI were significantly decreased, suggestive of cellular elastinopathy. Notably, induction of reactive astrocytosis by *Loxl1*-independent means resulted in almost identical molecular signatures.

Conclusions: *LOXL1* variants associated with exfoliation glaucoma may sensitize the optic nerve head to the deleterious consequences of elevated IOP by inducing reactive astrocytosis and cellular elastinopathy. Our data tentatively suggest a conserved a mechanism underlying reactive astrocytosis in exfoliation glaucoma and other subtypes of open angle glaucoma.

Poster #10 AAV delivery of alternative splice product of Complement factor H (CFH), FHL-1 to liver affects vision in *Cfh* knockouts.

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Purpose: Variants of Complement factor H (CFH) and its alternative splice form, FHL-1, are major genetic risk factors for AMD but the relative role/contribution of each form to AMD development is unknown. We used AAVs expressing FHL-1 to examine the function of FHL-1 in *Cfh* knock out (Cfh-/-) mice. We tested the hypothesis that FHL-1 directly contributes to AMD risk.

Methods: AAV vectors were designed for systemic expression of FHL-1, using a liver-specific promoter and delivered by tail vein injection in *Cfh-/-* mice. FHL-1 expression was measured on Westerns, and complement activity was assessed by Western blot of complement proteins C3, FB, and C5. FHL-1's effect on ocular phenotypes was assessed in aged (>90 weeks) *Cfh-/-* mice fed a high fat, cholesterol-enriched (HFC) diet (*Cfh-/-*~HFC). Visual function was measured by electroretinography (ERG).

Results: *Cfh-/-* mice injected with the AAV constructs had detectable levels of FHL-1 in their plasma. Plasma concentrations of C3 and FB were unchanged by FHL-1 expression. An increase in uncleaved C5 was detectable in young, but not aged, *Cfh-/-* mice fed an HFC diet. Visual function of *Cfh-/-*~HFC was affected by expression of FHL-1, resulting in an ERG b-wave deficit normally absent in this genotype.

Conclusions: FHL-1 expressed in the liver affects systemic levels of intact C5, indicating functionality of the construct. Development of a visual deficit with liver expression of FHL-1 supports a role of circulating FHL-1 in the development of phenotypes in our mouse model, which has implications for AMD therapies targeting the complement system.

Poster #11 Astrocyte Subtypes Determine the Fate of Ganglion Cells

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Purpose: Endothelin-1(ET-1) and its receptors are involved in the etiology of glaucoma. Previously, we reported that ET-1 treatment induced reactivation of astrocytes (ASTs) and apoptosis of retinal ganglion cells (RGC). However, ET-mediated subtype changes of ASTs and their effect on RGC survival are largely unknown. This study aimed to studying the roles of ET-1 in the interaction between RGCs and AST subtypes.

Methods: Primary rat RGCs were isolated from rat pup retinas and ASTs from optic nerve. Contact and non-contact RGC-AST co-cultures were treated with ET-1. ET-1-mediated intracellular calcium was monitored in RGCs, ASTs and co-culture of RGCs and ASTs using Fura-2 AM calcium imaging. Cell death and survival was detected using LIVE/DEAD assay. Expression of astrocytic markers in cultured ASTs and rat retina was determined by immunocytochemistry.

Results: ET-1-induced elevation of $[Ca^{2+}]_i$ was significantly attenuated in co-culture of RGCs and ASTs, and accordingly less cell death was also observed in both co-culture systems. More synapse formation was detected in RGC-AST co-cultures. The longer co-culture time produced more synapses. ET-1 treatment or c-Jun overexpression in ASTs changes the gene and protein expression profile of astrocytic markers. ET-1 intravitreal injection in rats also upregulated GFAP, Serping1 and CD14.

Conclusions: ASTs co-cultured with RGCs stimulate more synapse formation in RGCs and decrease RGC death. ET-1 treatment induces not only the reactivation of ASTs but also the switch of astrocyte subtypes, which could lead to dysfunction of axon transport in the optic nerve and affect RGC survival.

Poster #12 Dysregulation of ATX/LPA Signaling Axis in the Aqueous Humor of Human Glaucoma Patients

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Purpose: Lysophosphatidic acid (LPA), a bioactive lipid and product of autotaxin (ATX), has been shown to modulate the aqueous humor (AH) outflow and intraocular pressure (IOP). To seek possible etiological significance of dysregulated ATX/ LPA signaling axis in primary open angle glaucoma (POAG), here, we determined the levels of LPA and ATX in the AH of POAG patients and investigated the regulation of ATX expression in human primary trabecular meshwork (TM) cells.

Methods: The levels of ATX, LPA, and ATX substrate-lysophosphatidylcholines (LPC) in AH derived from POAG and cataract patients (n=25) were measured by ELISA and mass spectrometric analyses. The regulation of ATX protein and mRNA, in TM cells (n=6) using different physiological agents, siRNA and inhibitors was evaluated by immunoblot analysis.

Results: Significant increases in ATX protein levels was found in the AH of POAG patients compared to age and gender matched cataract patients and they exhibited a positive correlation with IOP values in POAG patients. Both LPA and LPC levels in the POAG samples were significantly elevated compared to cataract samples. ATX protein levels were significantly elevated in TM cells in response to the treatment with dexamethasone, TNF-α, and IL-1β via ERK, NF-κB or SMAD2/3, and glucocorticoid receptors. ATX deficiency and inhibition suppressed the fibrogenic activity in TM cells.

Conclusions: Collectively, these results reveal that the dysregulated levels of ATX, LPA and LPC in AH associate with the etiology of ocular hypertension in POAG patients, and ATX as a promising therapeutic target to lower IOP in glaucoma patients.

Poster #13 REV-ERBa Regulates RPE Function and Age-related Degeneration in Mice

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Purpose: Retinal pigment epithelium (RPE) dysfunction and atrophy is observed in patients with dry form of age-related macular degeneration (AMD). This often leads to further degeneration of nearby photoreceptors, which ultimately causes blindness. Dysfunction of RPE cells during aging is associated with oxidative stress, dysregulated cellular metabolism and inflammation during aging. Here we investigated the role of the nuclear receptor REV-ERBa, a redox sensitive transcriptional regulator of metabolism and inflammation, in regulating RPE function in AMD pathogenesis.

Methods: REV-ERBa deficient (*Rev-erba*$^{-/-}$) mice and littermate wild type (WT) controls were analyzed at various time points during aging. Morphological features were analyzed using fundus imaging and RPE/choroid complex flat mounts and cross-sections. Ultrastructure of RPE, retina and Bruch's membrane were characterized by electron microscopy. RPE phagocytosis was measured by fluorescent microbeads uptake. Expression levels of *Rev-erba* and relevant metabolic and inflammatory genes were analyzed in isolated *Rev-erba*$^{-/-}$ and WT RPE.

Results: REV-ERBa expression levels were decreased with age in WT RPE. Aged *Rev-erba*$^{-/-}$ mouse eyes showed abnormal lipid-enriched subretinal lesions, substantially decreased visual function, patchy areas of RPE degeneration with disrupted tight junctions and decreased expression of junctional genes. Moreover, phagocytic activity was significantly diminished in isolated *Rev-erba*$^{-/-}$ RPE cells. *Rev-erba*$^{-/-}$ RPE also exhibited a decrease in metabolic gene expression and an increase in inflammatory gene expression including complement components.

Conclusions: Our findings suggest that REV-ERBa is a novel regulator of RPE function and degeneration in AMD pathogenesis, and may serve as a potential molecular target for developing future therapeutics.

Poster #14 Aberrant BMAL1 dependent claudin-5 cycling induces geographic atrophy

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Purpose: Circadian rhythms involvement in retinal function is not fully understood. Here, we examined the role of circadian rhythms in the regulation of the inner blood-retinal barrier (iBRB) function that we show plays a role in the development of geographic atrophy (GA), the end stage of 'dry' age-related macular degeneration (AMD).

Methods: iBRB integrity was characterised *in vitro* by western blotting and qPCR and *in vivo* using fundus fluorescein angiography (FFA) and contrast enhanced magnetic resonance imaging. Mice were sub-retinally injected with adeno-associated virus targeting claudin-5 expression and placed onto a cholesterol-enriched diet. Eyes were enucleated and immunohistochemical analysis undertaken.

Results: Retinal claudin-5 expression is tightly regulated by *BMAL1* and the circadian clock leading to phenotypically more permeable retinal vessels in the evening. Persistent suppression of claudin-5 expression in mice exposed to a cholesterol-enriched diet induces retinal pigment epithelium (RPE) cell atrophy. A similar phenotype is observed in non-human primates where persistent claudin-5 suppression in the macular region induced RPE atrophy. FFA in human subjects showed increased retinal vascular permeability in the evening compared to morning.

Conclusion: Our findings show the iBRB is highly dynamic and implicates an inner retina derived component to the early pathophysiological changes observed in AMD. Circadian regulation of claudin-5 facilitates material exchange between blood and the neural retina allowing replenishment of 'spent' photoreceptor outer segments by the RPE. These results suggest re-establishing claudin-5 cycling at the iBRB may represent a novel therapeutic target for the prevention and treatment of GA secondary to dry AMD.

Poster #15 Schlemm's canal imaging, pressure measurement, catheterization and substance delivery in live monkeys

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Purpose

1) We describe a real time technique to measure pressure in Schlemm's canal (SC) that may help understanding glaucoma pathophysiology and evaluating potential therapeutics.

2) We describe a gene delivery technique allowing injection of smaller volumes and lower titers directly into SC, minimizing off-target effects.

Methods: 1) A needle, connected to a pressure transducer and computer, is placed into the anterior chamber to continuously record and control IOP. Concurrently, SC is imaged using an endoscope attached to a digital camera in turn connected to a computer. The images obtained are time synchronized with the collection of IOP data. 2) With gonioscopic/microscopic visualization of SC, a microcatheter is inserted into the canal circumferentially ab interno. The vector along with trypan blue dye is injected while withdrawing the catheter and visualized in the SC with an endoscope over 360°.

Results: 1) SC fills with venous blood and blanches as IOP is lowered and raised, respectively. The range of IOP over which these changes are noted is wider and higher than predicted (~5-22mmHg). Sausaging of the blood column in SC is observed indicating segmental differences. 2) 360° catheterization of SC was routinely executed. The vector was injected and visualized with an endoscope as a bright blue band within SC.

Conclusion

1) Recording canalicular morphology and IOP, with synchronized time stamping, will provide a precise description of pressure-related canalicular dynamics.

2) Placing a microcatheter directly into SC may facilitate development of gene therapies targeting conventional outflow pathway structures.

Poster #16 Role of miR-29c-3p in regulation of extracellular matrix synthesis

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Purpose: To investigate changes in microRNA (miRNA) expression in lamina cribrosa (LC) cells treated with transforming growth factor beta 2 (TGFβ2).

Method: Non-glaucomatous primary human LC cells were grown to 100% confluency and treated with TGFβ2 (5ng/ml) or control for 24hours. Differences in expression of miRNAs were analysed by miRNA Q-PCR array. LC cells were transfected with miR-29c-3p (10nM) mimic, inhibitor or non-targeting controls and analysed by Q-PCR to confirm overexpression or knockdown of miR-29c-3p. mRNA targets of miR-29c-3p were determined through protein expression analysis by immunocytochemistry. The effects of miR-29c-3p and TGFβ2 on collagen type (COL) I and IV protein expression were evaluated in cells transfected with miR-29c-3p mimic, inhibitor or control and treated with TGFβ2 expression.

Result: miRNA PCR arrays showed that TGFβ2 treatment downregulated the expression of miR-29c-3p in LC cells. Transfection of miR-29c-3p mimic or inhibitor showed upregulation and downregulation of miR-29c-3p respectively, confirming transfection efficiency. Immunocytochemistry analysis showed that miR-29c-3p regulates the expression of COL I and IV. Overexpression of miR-29c-3p decreased TGFβ2 induced COL I and IV expression. Inhibition of miR-29c-3p exacerbated the effects of TGFβ2 on COL I and IV expression.

Conclusion: TGFβ2 induced the downregulation of miR-29c-3p, an anti-fibrotic miRNA, which may stimulate a pro-fibrotic response and pathogenic remodelling of the optic nerve head. The inhibitory effects of miR-29c-3p on TGFβ2, suggest that miR-29c-3p regulates ECM protein synthesis and restoring the expression of miR-29c-3p may preserve the microarchitecture of the LC.

Poster #17 Sodium 4-phenylbyrate rescues glucocorticoid-induced ocular hypertension by reducing abnormal ECM deposition in the TM via activation of MMP9

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Purpose: Ocular hypertension (OHT) is a serious side effect of glucocorticoid (GC) therapy and if left undiagnosed, it leads to glaucoma. Previously, we have shown that, sodium-4-phenylbutyrate (PBA) rescues GC-induced glaucoma. However, the exact mechanism behind PBA mediated reduction of GC-induced OHT is not completely understood. Here, we examined whether PBA rescues GC-induced OHT by reducing abnormal ECM deposition via activating matrix metalloproteinases (MMP).

Methods: GC-induced OHT mice were topically instilled 1% PBA or vehicle twice daily. IOPs were measured every week and outflow facility was analyzed after 5-weeks of treatment. Immunostaining, Western blot and qPCR were performed to analyze the effect of PBA on Dex-induced ECM deposition and ER stress in primary human TM cells, mouse and ex-vivo cultured human TM tissues. Zymography used to study the effect of PBA on MMP activity.

Results: Topical PBA (1%) eye-drops reduced GC-induced OHT and also improved outflow facility significantly. PBA reduced GC-induced intracellular and extracellular ECM accumulation and also prevented induction of ER stress in primary human TM cells, ex-vivo human cultured TM tissues and mouse TM tissues. Interestingly, we observed that PBA can reduce existing ECM deposition when primary TM cells were grown on decellularized GC-treated ECM. Gene expression, Western blot and zymography assays revealed that PBA induces increased expression and activity of MMP-9 over control in glaucomatous cells. Inhibition of MMP-9 activity by chemical-inhibitors abrogated PBA's effect on ECM reduction.

Conclusions: PBA can be a very attractive treatment for general POAG via targeting abnormal ECM accumulation in the TM.

Poster #18 Vitamin B12 deficiency caused by metformin is associated with the risk of diabetic retinopathy

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Purpose: Metformin is the cornerstone drug treatment for type 2 diabetes. Recent studies show that this treatment is associated with deficiency of vitamin B12, which is thought to be caused by impaired vitamin B12 absorption. The aim of this study was to evaluate the associated risk of the vitamin B12 deficiency in metformin-treated patients with and without diabetic retinopathy.

Methods: We enrolled 384 diabetic retinopathy patients treated for at least 3 years with metformin and 170 diabetic patients managed with diet or insulin or other treatments. Circulating vitamin B12 levels along with total cholesterol, HDL cholesterol, triglycerides, glycated hemoglobin, homocysteine and folate were evaluated. Prevalence, predictive values, sensitivity and specificity were assessed by Bayes' theorem.

Results: Vitamin B12 deficiency in metformin-treated patients with diabetic retinopathy showed positive predictive value 85%, negative predictive value 70%, sensitivity 87%, specificity 67%, prevalence 69%.

Conclusions: Long term of metformin treatment causes vitamin B12 deficiency that is associated with the risk of diabetic retinopathy. Therefore, we suggest that vitamin B12 supplementation could be useful in patients under long-term metformin treatment.

Poster #19 Assessment of soluble epoxide hydrolase and role of lipid mediators in choroidal neovascularization

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Purpose: Choroidal neovascularization (CNV) is a major pathological feature of age-related macular degeneration (AMD), and new therapies are still needed. Previously, we identified soluble epoxide hydrolase (sEH) as a binding target of an antiangiogenic small molecule, SH-11037. Here, we aimed to investigate sEH expression in the eye and the effects of the lipid substrate and product of sEH in CNV.

Methods: The expression localization, and activity of sEH in eyes of laser-induced choroidal neovascularization (L- CNV) mice were evaluated by immunohistochemistry and an ex vivo enzymatic assay. The sEH inhibition by SH-11037 and mode of inhibition was assessed by recombinant sEH activity assay. Also, sEH substrate 19,20-epoxy docosapentaenoic acid (EDP) and product 19,20-dihydroxy docosapentaenoic acid (DHDP) were injected intravitreally in L-CNV mice, and effects were assessed by imaging and immunofluorescence.

Results: sEH was upregulated in the rod photoreceptors in L-CNV mice compared to control mice and it did not co- localize with other retinal cell type markers. Correspondingly, sEH activity was increased in L-CNV eyes and was normalized by SH-11037 or a known sEH inhibitor. SH-11037 inhibited recombinant sEH activity, with IC_{50} = 0.15 µM. Enzyme kinetics analysis demonstrated that SH-11037 decreased V_{max} and increased K_M, revealing SH-11037 as a mixed-type inhibitor of sEH, with K_i = 1.73 ± 0.45 µM. Intravitreal 19,20-EDP reduced CNV lesion volume compared to vehicle and 19,20-DHDP.

Conclusions: Our studies confirm the relevance of sEH and its lipid metabolites for choroidal neovascularization, which these mediators affect angiogenesis will be critical.

Poster #20 Activation of TRPV4 channels reduces IOP and improves outflow facility by regulating eNOS dependent NO release from the TM

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Purpose: Nitric oxide (NO) is known to reduce intraocular pressure (IOP) by relaxation of the trabecular meshwork (TM) and distal vessels of the conventional outflow pathway. Nonetheless, the intrinsic pathways by which outflow pathway tissues regulate NO production is yet to be elucidated. In vascular endothelium, activation of mechanosensory transient receptor potential vanilloid 4 (TRPV4) channels results in endothelial nitic oxide synthase (eNOS) mediated NO release, which in turn promotes vasodilation. Here, we explored whether TRPV4 activation regulates NO release in the conventional outflow pathway.

Methods: In wildtype and glucocorticoid-induced ocular hypertensive C57BL/6J mice, the effect of TRPV4 agonist GSK1016790A on IOP and outflow facility was determined using rebound tonometry and constant-flow infusion method respectively. Effect of TRPV4 agonist on eNOS activation and NO production was determined using Western blot and DAF-FM assay in primary human TM cells and *ex vivo* cultured human TM donor tissues. We developed a novel method for electrochemical measurement of NO using NO electrodes in human anterior segment tissues.

Results: Topical administration of TRPV4 agonist significantly reduced IOP and increased outflow facility. TRPV4 activation increased eNOS phosphorylation and NO production in primary human TM cells and *ex vivo* cultured human TM. Treatment of human anterior segments with TRPV4 agonist resulted in increased production of NO as detected electrochemically. Nonselective inhibition of NOS by L-NAME abrogated the IOP lowering effect of TRPV4 agonist in mice and reduced NO production in outflow pathway cells and tissues.

Conclusion: TRPV4 activation improves IOP and outflow, perhaps by NO regulation.

Poster #21 Non-invasive monitoring of suprachoroidal, subretinal and intravitreal biodegradable implants using confocal laser scanning ophthalmoscope (cSLO) and optical coherence tomography (OCT)

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Purpose: Our long-term goal is to facilitate continuous noninvasive monitoring of drug delivery in the eye. Although injectable implants are reality for the vitreous humor, we are not aware of similar efforts in the suprachoroidal and subretinal spaces. To address these gaps, our objective was to inject implants into suprachoroidal, subretinal, and intravitreal spaces and monitor their length, diameter, and volume noninvasively.

Methods: Biodegradable poly(lactide-co-glycolide) (PLGA) implants of various lengths and diameters were injected in isolated bovine eyes at suprachoroidal, subretinal, and intravitreal locations. The implants were imaged noninvasively using cSLO and OCT modes of Heidelberg Spectralis HRA+OCT instrument after adjusting the corneal curvature for the bovine eye.

Results: Simultaneous cSLO and OCT images identified implants in different regions. Implant length and diameter were obtained using cSLO images. Volumes for suprachoroidal and subretinal implants were estimated by integrating the bleb areas, at various depths in OCT images or by using the thickness map of 1 mm diameter central circle of ETDRS grid. Intravitreal implant volume was estimated using the dimensions obtained from cSLO images. Image-based measurements of length, diameter, and volume correlated well with the values prior to injection. The accuracy for noninvasive measurement of PLGA implant for length, diameter, and volume ranged from 93-104%, 75-118%, and 58-171%, respectively, for the various routes and approaches.

Conclusions: Suprachoroidal, subretinal, and intravitreal implants can be monitored for their length, diameter, or volume using cSLO and OCT imaging. Such measurements may be useful in monitoring implant degradation and drug release in vivo.

Poster #22 Computational systems biology approach to identify novel pharmacological targets for diabetic retinopathy

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Purpose: Diabetic retinopathy was included by the World Health Organization in the eye disease priority list. Up to now, only proliferative diabetic retinopathy can be treated with approved drugs, such as intravitreal anti-vascular endothelial growth factor (VEGF) agents or steroids. In this perspective, there is the urgent need to explore novel pharmacological targets for treatment of diabetic retinopathy.

Methods: We applied a systems biology approach to identify novel drug targets for diabetic retinopathy. Transcriptomic data were retrieved from Gene Expression Omnibus Dataset repository (GEO) datasets. Analysis of GEO datasets was carried out with an enrichment-information approach, which gave as output a series of complex gene-pathway and drug-gene networks. Networks were analyzed through Cytoscape. Bioinformatic data have been integrated with literature search and data mining through clinicaltrial.gov.

Results: Bioinformatic analysis predicted 102 putative pharmacological targets. No current references are available regarding 19 predicted targets, while, only 15 targets have been investigated in clinical trials for treatment of diabetic retinopathy. Our computational analysis has revealed 19 novel potential pharmacological targets for diabetic retinopathy: *SLC13, CFLAR, SCTR, FSHB, RLN3, ADR1D, ADR2C, CHRM5, LPAR2, FSHR, THRB, GLRA3, PRSS3, KCNJ16, KCNE2, ATP4A, ATP1B4, ADCY7, PTGES.*

Discussion: Analysis of these networks identified genes and biological pathways related with inflammation, fibrosis and G protein-coupled receptors that are potentially involved in development of the disease. This analysis provided new clues on novel pharmacological targets, useful to treat diabetic retinopathy.

Poster #23 Crosstalk Between Transforming Growth Factor Beta-2 and Toll Like Receptor 4 in the Trabecular Meshwork

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Purpose: One of the primary risk factors for primary open angle glaucoma (POAG) is elevated intraocular pressure (IOP). Discovering potential new targets to lower IOP is necessary for effective drug therapies. We recently discovered a novel molecular mechanism involved in the development of glaucomatous trabecular meshwork (TM) damage and elevated IOP. We have identified TGFβ2 and toll-like receptor 4 (TLR4) signaling crosstalk regulates changes in the TM ECM and mutation in *Tlr4* rescues TGFβ2 -induced ocular hypertension in mice. Here, we investigated the role of an endogenous TLR4 ligand, FN-EDA, and a downstream signaling molecule, NFκB, in TGFβ2-induced ocular hypertension in mice.

Methods: B6.FN-EDA$^{-/-}$, B6.FN-EDA$^{+/+}$/TLR4$^{-/-}$, B6.FN-EDA$^{-/-}$/TLR4$^{-/-}$, and C57BL/6J mice were intravitreally injected with 2.0µL Ad5.TGFβ2 (2.5x10^{7}pfu) in one eye and the contralateral eye was uninjected. Likewise, we tested mice lacking the p50 subunit of NFκB (B6.Cg-*NFκB1tm1Bal*/J) and C57BL/6J mice. IOP was measured once per week using a rebound tonometer on isoflurane-anesthetized mice. Significance determined by one-way ANOVA at each time point. At 6-week post-injection eyes were harvested, fixed, and sectioned for immunohistochemistry to assess total fibronectin and FN-EDA isoform expression.

Results: Ad5.TGFβ2 significantly induced ocular hypertension in C57BL/6J mice. Mutations in *Tlr4, FN-EDA*, and *NFκB* blocked Ad5.TGFβ2 induced ocular hypertension with no significant IOP elevation. Total FN and FN-EDA isoform expression increased in Ad5.TGFβ2 injected C57BL/6J mice.

Conclusions: TLR4, FN-EDA, and NFκB are necessary for TGFβ2 induced ocular hypertension in mice. These data provide potential new targets to lower IOP.

Poster #24 Identification of the functional complex between hnRNPL and the glaucoma-associated long noncoding RNA, PEXpress

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Purpose: Pseudoexfoliation (PEX) glaucoma is a common and aggressive form of glaucoma with distinct clinical manifestations of fibrillary material deposition in the conventional outflow pathway. The long noncoding RNA, *PEXpress*, is highly associated with risk of PEX glaucoma, however the mechanism by which it regulates molecular changes in the tissues of the outflow pathway is unknown.

Methods: Fluorescence in-situ hybridization probes targeted to *PEXpress* localized *PEXpress* subcellularly. Interacting proteins were identified using a streptavidin pulldown assay with biotinylated *PEXpress* in conjunction with mass spectrometry. RNA-seq identified and quantified downstream gene targets of *PEXpress*. Adenovirus encoded with targeted shRNAs was used to knock down *PEXpress* in Schlemm's canal (SC) cells. Signaling molecules AKT, MAPK and FAK were analyzed for their phosphorylation status by Western blot, and cellular morphology was assessed in SC cells using Nikon software.

Results: *PEXpress* localized predominantly to the nuclei of cells and bound the mRNA regulatory protein, hnRNPL. In immortalized human lens (HLE-B3) cells, knockdown of *PEXpress* led to dysregulation of over 450 target genes, while overexpression of *PEXpress* significantly altered the expression of ~100 other genes. When *PEXpress* abundance was manipulated, phosphorylation status of MAPK, AKT and FAK was not changed in HLE-B3 cells. Interestingly, knockdown of *PEXpress* in SC cells led to a significant increase in the ratio of pAKT/AKT, which was coincident with a significant change in cellular morphology.

Conclusions: *PEXpress* regulates gene expression, AKT signaling, and cellular morphology in glaucoma relevant cells, making it a viable therapeutic target for PEX glaucoma.

Poster #25 Assessment of ocular blood flow regulation using laser speckle flowgraphy in healthy subjects

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Purpose: Ocular blood flow (OBF) regulation during changes in ocular perfusion pressure (OPP) or during stimulation with flicker light has been extensively investigated in the recent years using various techniques. The aim of the present studies was to evaluate whether Laser speckle flowgraphy (LSFG), a commercially available technique for measuring OBF, is capable to assess blood flow regulation.

Methods: In 27 subjects, measurements of optic nerve head (ONH) and retinal blood flow were performed during isometric exercise and in 20 subjects, blood flow was assessed before and during stimulation with flicker light. Mean blur rate (MBR) in the ONH (MBR_{ONH}), relative flow volume (RFV) in retinal arteries (RFV_{ART}) and veins (RFV_{VEIN}) were assessed using LSFG.

Results: In the isometric exercise experiments, the relative increase in OPP (78.5 ± 19.8%) was more pronounced than the increase in OBF parameters (MBR_{ONH}: 18.1 ± 7.7%, RFV_{ART}: 16.5 ±12.0%, RFV_{VEIN}: 17.7 ± 12.4%) indicating for an autoregulatory response of the vasculature. Retinal stimulation with flicker light induced a significant increase in MBR_{ONH} by +17.5%±6.6% ($p<0.01$), as well as in RFV_{ART} by +23.8±10.0% ($p<0.05$) and in RFV_{VEIN} +23.1%±11.0 ($p<0.05$).

Conclusions: As expected, the increase in OBF parameters was less pronounced than the increase in OPP during isometric exercise, while flicker stimulation induced a significant increase in OBF. These results indicate that LSFG is an appropriate method for the quantification of retinal and ONH blood flow during different provocation tests and may be applied as a non-invasive, easy to use tool to assess OBF regulation in humans in the future. Support from the Austrian Science Fund FWF KLI 529 is gratefully acknowledged.

Poster #26 Characterization of RPE Cell Death Induced by 4-HNE

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Purpose: Degeneration of the retinal pigment epithelial (RPE) underscores the pathology in geographic atrophy (GA), a late stage dry age-related macular degeneration (AMD). Oxidative stress and aging are known to contribute to GA pathogenesis. However, how RPE cells die in GA is still controversial. 4-Hydroxynonenal (4-HNE) is an oxidative stress marker produced by lipid peroxidation, which is accumulated in aging cells and could be related to age-related diseases. The goal of the study is to determine the mechanism of 4-HNE-induced RPE cell death.

Methods: ARPE-19 cells were treated with 4-HNE at different concentrations to induce cell death**.** Inhibitors of apoptosis, necroptosis, pyroptosis and ferroptosis pathways, were used to test for their effect on cell viability. Cell morphology and molecular markers (including PYCARD and RIPK3) were examined under a microscope. ATP and ROS levels were measured using standard methods.

Results: We found that 4-HNE (5-10 ug/ml) induces significant RPE cell death, consistent with significantly decreased cellular ATP but increased ROS levels. Necrosis inhibitor Necrostatin-7 and ferroptosis inhibitors liproxstatin-1 and DFO, but not apoptosis inhibitors, prevented 4-HNE-induced RPE cell death. On the molecular level, both inflammasome and necrosome are activated in RPE cells treated with 4-HNE, as shown by PYCARD and RIPK3 visualization.

Conclusions: Ferroptosis and/or necroptosis, but not apoptosis, underlie RPE death induced by 4-HNE. Crosstalk between different cell death pathways may occur when RPE cells are under oxidative stress. The inhibitors from our study may have therapeutic implications for GA.

Poster #27 Cathepsin K, a secreted cysteine protease, regulates transforming growth factor-β2 bioavailability and extracellular matrix in the trabecular meshwork

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PURPOSE: Cysteine protease like Cathepsin K (CTSK) regulates extracellular matrix (ECM) remodeling. CTSK degrades helical and non-helical regions of collagen 1 and regulates transforming growth factor-β(TGFβ) stability. We investigated the role of CTSK on TGFβ2 bioavailability and ECM remodeling in human trabecular meshwork (HTM).

METHODS: CTSK expression, activity and distribution in HTM cell, and in aqueous humor (AH) outflow tissue was assessed. Effects of adenovirus-mediated expression of CTSK (AdCTSK) on changes in actin organization, ECM production and remodeling, and TGFβ2 levels were analyzed using qPCR, immunofluorescence, and immunoblotting. Students t-test was used for statistical analyses and results were significant if $p<0.05$ with a sample size of $N\geq3$ in each experiment.

RESULTS: CTSK is distributed in the TM and juxta-canalicular tissues of AH outflow pathway and CTSK activity is found in HTM cell extracts and culture media. Increasing CTSK expression in HTM cells using AdCTSK resulted in- a) shorter and coalesced actin stress fibers assessed using phalloidin staining, b) decreased collagen-1A and fibronectin assembly and fibril formation as visualized by immunofluorescence, and c) significant decrease in collagen-1A and fibronectin expression ($p<0.05$, $n=3$). AdCTSK significantly decreased TGFβ2 mRNA ($p=0.004$, $n=3$) and total intracellular TGFβ2 levels ($p<0.05$, $n=3$).

CONCLUSION: Together, these preliminary findings identify CTSK as an important ECM modulator in the TM. We believe that CTSK is involved in the IOP homeostasis by regulating the bioavailability of TGFβ2 and maintaining optimal cell-matrix interactions in the AH outflow pathway. Any kind of dysregulation in CTSK functions can lead to elevated IOP.

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Poster #28 Intravenous treatment of choroidal neovascularization by photo-targeted nanoparticles

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ABSTRACT: Choroidal neovascularization (CNV) is the major cause of vision loss in wet age-related macular degeneration (AMD). Current therapies require repeated intravitreal injections, which are painful and can cause infection, bleeding, and retinal detachment. We developed a drug delivery system that can be administered intravenously and accumulate in the back of the eye by light-triggered targeting. Photo-targeted nanoparticles (NP-[CPP]) were formed from PEG-PLA chains modified with cell penetrating peptide (CPP). Cell uptake of NP-[CPP] was inactivated by attaching a photocleavable group DEACM to the CPP, which also placed [CPP] in the core of the nanoparticle, preventing it from interacting with cells. Irradiation with 400 nm (blue) light cleaved DEACM, releasing CPP from the NP core and rendering it active. This system was evaluated in mice with laser-induced CNV. After intravenous injection of NP-[CPP], irradiation at the eye cleaved DEACM, allowing NP accumulation in the choroidal neovascular lesions. NP- [CPP] with irradiation showed greater accumulation in neovascular lesions compared to the same nanoparticles without irradiation or nanoparticles without CPP. In the same mouse CNV model, NP-[CPP] loaded with doxorubicin significantly reduced neovascular lesion size. This phototriggered targeting strategy could allow non-invasive treatment of CNV and similar diseases, and enhance the proportion of drug in diseased areas of the eye vs. other healthy parts of the eye or body.

Poster #29 Does protein acetylation plays crucial role in δ-opioid receptors mediated retinal ganglion cell neuroprotection in glaucoma?

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Purpose: This study determines the role of protein acetylation and its downstream targets that play crucial role in the δ-opioid receptor agonist, SNC-121-mediated retina neuroprotection in glaucoma model.

Methods: Intraocular pressure (IOP) was raised in Brown Norway rats by injecting 2M hypertonic saline into the limbal veins. Animals were administered with SNC-121 (1 mg/kg; i.p) daily for 7 days. Retinas were determined for the changes in the expression of genes and proteins at day 7^{th} using qPCR based array and Western blotting.

Results: IOP was increased significantly ($P<0.05$) in ocular hypertensive (OH) animals. Elevated IOP caused a significant increase in HDAC (Histone Deacetylase) Class I (HDAC 1, 2, 3 and 6) activity by 17.8±5% ($P<0.05$) that was abrogated by SNC-121 treatment. Concomitantly, acetylated histone H3 levels were decreased in OH retinas and restored by SNC-121 treatment. A significant ($p<0.05$) increase in mRNA expression of transcriptional factors STAT3 and decrease in CREB ($p<0.05$) was observed OH animals, which was restored to normal levels by SNC-121 treatment. SNC-121 phosphorylated CREB whereas, SNC-121 inhibited phosphorylation of STAT3 in the OH animals. Additionally, a significant decrease (> 2fold, $P<0.05$) in level of neurotrophic factors (e.g., BDNF, CNTF, and FGF) and increase in pro-inflammatory cytokines (e.g., IL-1β and IL-6) was seen in the retina of OH animals.

Conclusions: Our data suggest that IOP exacerbation disrupts protein acetylation homeostasis, favoring the production of pro-inflammatory milieu in OH eye. Our novel finding suggests that early intervention with SNC-121 reverse such changes, thereby providing neuroprotection against glaucomatous injury.

Poster #30 A guinea pig-based screening of intraocular pressure lowering drugs as a novel avenue for controlling myopia progression

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Purpose: In an already published study, we reported that topical latanoprost was effective in both inducing a sustained (across 24 h) decrease in IOP and slowing myopia progression in myopic guinea pigs. As the first step in a follow-up study, this study examined the efficacy of four different glaucoma drug classes in lowering intraocular pressure in guinea pigs as potential alternative avenues for controlling myopia progression.

Methods: 7-9 months old guinea pigs (GPs) underwent monocular topical glaucoma drug treatment for 4 weeks. 15 GPs were equally divided into five treatment groups of 3 animals that received: 1) Latanoprost QD (Prostaglandin, 0.005%), 2) Timolol QD (Beta blocker, 0.5%), 3) Brimonidine BID (a2 agonist, 0.15%), 4) Brinzolamide suspension BID (Carbonic anhydrase inhibitor, 1%), and 5) Non-preserved artificial tears QD (control). Diurnal intraocular pressures (IOPs) were recorded at baseline (before treatment), and after 2 and 4 weeks of treatment, in each case at 4 time points, using iCare tonometry.

Results: Key results are summarized in Table 1, as changes in interocular differences from baseline. Both latanoprost and timolol induced a sustained lowering of IOP, as reflected in changes recorded at week 4. Brimonidine and Brinzolamide were not as effective in lowering IOP.

Conclusions: As shown previously for latanoprost, daily topical timolol was also effective in lowering IOP over 24 h in healthy guinea pigs. This result contrasts with previous human studies, which reported timolol (sympathomimetic) to be ineffective in lowering IOP during the night. This may reflect the crepuscular nature of guinea pigs.

Table1: Mean interocular (treated-control) differences; ± SEM in mmHg at the end of the treatment (4 weeks) compared to baseline.

Treatment group	9:35 am	3:35 pm	9:25 pm	3:25 am	Grand mean
Artificial tears	3.8 ± 1.45	-3.0 ± 0.12	2.1 ± 0.43	-1.1 ± 0.75	0.45 ± 0.69
Latanoprost	0.22 ± 2.6	-4.7 ± 1.43	-1.4 ± 0.58	-2.6 ± 0.02	-2.12 ± 1.16
Briminodine	-0.44 ± 0.29	2.0 ± 0.49	0.0001 ± 0.77	3.8 ± 1.7	1.34 ± 0.81
Brinzolamide	2.7 ± 3.08	2.3 ± 0.45	-2.0 ± 0.4	-0.22 ± 0.7	0.70 ± 1.16
Timolol	-2.8 ± 0.4	-0.11 ± 1.24	0.22 ± 0.35	-3.7 ± 2.42	-1.60 ± 1.10

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Poster #31 Using Tandem-Mass-Tags to Quantify Sex-Dependent Retinal Proteome Phenotypes Identified by Electroretinography

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Purpose: A wide array of biological and technical variables can affect electroretinogram (ERG) outcomes, including gender. In turn, the prevalence of many retinal diseases exhibit strong gender dependencies. When we examined mouse scotopic ERG data by sex, trends were obvious under resting conditions. Therefore, we used a mass spectrometry (MS) based approach to characterize potential sex-based differences in the retina of the normal adult mouse.

Methods: 30-week-old outbred Swiss-Webster ND4 mice were used. Scotopic ERGs were obtained in 8 male and 8 female ketamine-xylazine anesthetized mice. Retinae from 5 male and 5 female mice were processed for MS3 analysis on a Fusion Orbitrap Mass Spectrometer (Thermo Fisher) using Tandem-Mass-Tags for quantification. Statistical analysis was performed using Stata$_{SE15}$, and bioinformatics by Ingenuity Pathway Analysis (Qiagen).

Results: Male mice have significantly higher ERG b-wave amplitudes. MS identified 4,264 proteins, 4,093 of which were quantified. 68 proteins were differentially expressed, between genders, by at least 1.5-fold and 32 of those by at least 2-fold. Two identified pheromone proteins, previously unreported in retina, differed more than 4-fold by gender. Additionally, 17 crystallin proteins were quantified, 12 of which were differentially expressed, with 10/12 more abundant in males.

Conclusions: Distinct molecular phenotypes likely contribute to the significantly greater resting scotopic ERG amplitudes observed in male mice, given that the expression levels of a large number of proteins involved in structure, development, signaling, and metabolism exhibited sex-dependent differences. Our findings may provide phenotypic insights into retinal function, as well as why many retinal diseases exhibit unique gender disparities.

Poster #32 Stabilization of hypoxia-inducible transcription factor-1a (HIF-1a) in rat glaucomatous optic nerve

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Purpose: This study was designed to determine the stabilization of hypoxia-inducible transcription factor-1a (HIF-1a) and production of pro-inflammatory cytokines in rat glaucomatous optic nerves.

Methods: Brown Norway rats were used to elevate intraocular pressure (IOP) by injecting 50 µL of 2 M hypertonic saline into the circumferential limbal veins. IOP was recorded prior to surgery (baseline IOP) and weekly after injury. HIF-1a inhibitor (KC7F2 0.05- 2 mg/kg; i.p.) was administered right after injury and subsequently daily for 28 days. The changes in the level of pro-inflammatory cytokines, GFAP, and HIF-1a were measured by RT-PCR, Western blotting, and immunohistochemistry.

Results: Intraocular pressure (IOP) elevation stabilizes HIF-1a at day 7th and 28th, post ocular injury. HIF-1a protein expression was increased by 45±4 (P<0.05) and 75±7 (P<0.05) in the optic nerve of ocular hypertensive animals at day 7th and 28th, respectively. Both HIF-1a stabilization and GFAP staining (glial cell activation marker) were increased and co-localized in optic nerves of ocular hypertensive eyes. Additionally, pro-inflammatory cytokines (e.g. TNF-a, and IL-1β) were up-regulated in ocular hypertensive animals and their levels were reduced significantly (P<0.05) in a HIF-1a inhibitor (e.g., KC7F2) treated animals.

Conclusions: Our data suggest that glial cell activation, HIF-1a stabilization, and their co-localization occur within optic nerves in an early stage of glaucoma development. Glial cell-induced pro-inflammatory cytokines could be regulated by transcriptional factor like HIF-1a and that can be a potential therapeutic target for the glaucoma therapy.

Poster #33 Use of ultrasound biomicroscopy to improve assessment of outflow facility by tonography

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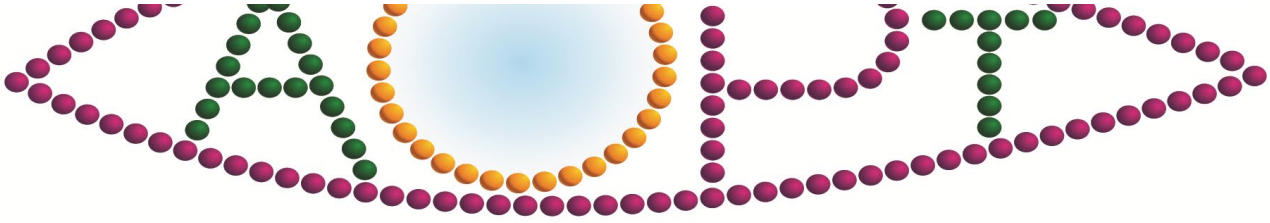
Purpose: Accurate assessment of outflow facility (C) is crucial for understanding glaucoma and treatment potential. C, is the ratio of a change in aqueous flow with change in intraocular pressure (IOP). The Friedenwald tables provide an estimate of aqueous flow change during the two-minute tonography procedure. This proof of concept experiment modifies the technique by utilizing ultrasound biomicroscopy (UBM) to measure individualized changes in aqueous flow.

Methods: Ten sedated rabbits were placed in lateral decubitus position. Tonography was performed for 2-minutes using a pneumatonometer with a 10-gram weight applied to the probe shaft and placed on the cornea. Serial images of the anterior chamber (AC) from nasal to temporal edges were captured with UBM before and after probe placement. Both eyes were measured. Five observers identified the posterior corneal and anterior iris surfaces as the borders of the AC in serial images. Algorithm-based reconstruction of the surfaces were used to determine AC volume and volume change during two minutes. Two-tailed t-tests compared IOP and AC volume, before and after tonography.

Results: The AC volume was 219.5±25.7 µL (mean±SD) before tonography and 211.8±22.3 µL after tonography (change of 7.7 µL, $p=0.03$). The aqueous flow was 3.6±4.3 µL/min. The IOP was 26.2±3.59 mmHg before tonography and 22.4±3.89 mmHg after tonography (change of 3.82 mmHg, $p<0.001$). C was 1.54±1.77 µL/mmHg.

Conclusions: UBM shows potential for measuring real-time AC volume changes during tonography. Automation, better border identification and faster image collection should make tonography a valuable tool for patient care and research purposes.



Poster #34 Activation of sigma 1 receptor (Sig1R) regulates NRF2 activity in cone photoreceptor cells

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Purpose: Sig1R is a novel target for treatment retinal degenerative diseases, but the mechanism is uncertain. We recently reported remarkable rescue of cone photoreceptor cell function when (+)-pentazocine ((+)-PTZ)), a high affinity Sig1R ligand was administered to *rd10* mice, a model of retinitis pigmentosa. *Rd10* degeneration is accompanied by significantly increased oxidative stress. In (+)-PTZ-treated *rd10* mice, levels of NRF2, a master regulator of the antioxidant response, were normalized and retinal oxidative stress decreased significantly, leading us to explore the role of Sig1R in modulating NRF2 activity and expression.

Methods: Sig1R was activated in 661W cone cells by exposure to (+)-PTZ [0-100μM] followed by assessment of: (A) oxidative stress (induced by tBHP) using CellROX; (B) viability (MTT assay); (C) NRF2 activation; (D) NRF2-KEAP1 binding; (E) NRF2 expression at the gene/protein level. The consequences on NRF2 expression when Sig1R was silenced using siRNA were investigated.

Results: We found that (+)-PTZ-mediated activation of Sig1R significantly attenuated tBHP-induced decrease in cell viability and increase in oxidative stress. (+)-PTZ-treated 661W cells showed an increase in NRF2-ARE binding activity, however (+)-PTZ did not directly inhibit KEAP1-NRF2 binding. (+)-PTZ-treatment led to an increase in NRF2 expression, both in whole lysates and nuclear extracts. Significantly decreased NRF2 levels and increased oxidative stress were observed in Sig1R-siRNA-661W cells.

Conclusion: (+)-PTZ does not directly inhibit KEAP1-NRF2 binding, however, its activation of Sig1R profoundly influences NRF2 expression and activity. Based on our findings, we hypothesize that a novel mechanism by which Sig1R activation mediates retinal neuroprotection is by modulating NRF2.

Poster #35 Decoding the anti-cataractogenic mechanism of grapes via a systemic pharmacology approach

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Introduction: Our previous study has shown that grapes could protect against *in vivo* ultraviolet B (UV-B) radiation-induced cataract. To better understand their mechanisms of action in cataract prevention, this follow-up study was designed to identify the molecular targets of grapes in the lens by using a systemic pharmacology approach.

Methods: As recommended by the California Table Grape Commission (CTGC), we selected four compounds including resveratrol, catechin, quercetin, and anthocyanins as the major phytoconstituents of grapes for target prediction. All genes that can be regulated by grapes were obtained from NCBI (www.pubmed.gov) and TCMSP (http://lsp.nwu.edu.cn/tcmsp.php). Genes that are associated with cataracts were collected from GeneCards (www.GeneCards.org). The comparison between grape-related targets and cataract-associated genes was conducted using Cytoscape 3.2.1 with ClueGo plugin. Gene Ontology (GO) enrichment analysis of grape-regulated genes was conducted using Database for Annotation, Visualization, and Integrated Discovery (www.david.ncifcrf.gov).

Results: A total of 332 targets that are regulated by grapes were identified and visualized by protein network. Subsequently, 147 GO functional pathways were clustered, including anti-apoptotic, anti-inflammatory, PI3K-Akt signaling, ATP binding, and FOXO pathways. Among these protein targets, X-linked inhibitor of apoptosis (XIAP), heat shock protein (HSP) 90, and prostaglandin-endoperoxide synthase (PTGS) were correlated with all of our selected phytoconstituents. Comparison between grape targets and cataract disease genes showed that 13 grape targets overlapped with cataract associated genes, including PTGS2, HSP90AA1, HSP90AA2P, mitogen-activated protein kinase 1 (MAPK1), MAPK14, MAPK3, amyloid precursor protein (APP), glycogen synthase kinase 3B (GSK3B), protein kinase a (PRKCA), protein kinase C delta (PRKCD), B-cell lymphoma 2 (BCL2), BCL2L1, and K-ras (KRAS).

Conclusions: The anticataractogenesis effects of grapes may encompass more than direct scavenging of free radicals but also activating the anti-apoptotic pathway.

Poster #36 Treatment of Pseudomonas aeruginosa infectious keratitis with photodynamic antimicrobial therapy (PDAT): Riboflavin and Rose Bengal

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Purpose: Infectious keratitis is a potentially blinding disease affecting patients worldwide. Photodynamic antimicrobial therapy (PDAT) is a novel, promising treatment for infectious keratitis. This study compares the clinical outcomes of two patients successfully treated with PDAT using two different photosensitizers: riboflavin and rose bengal.

Methods: Two patients presented with *Pseudomonas aeruginosa* keratitis spreading from limbus-to-limbus. Despite antibiotic management, both patients worsened in the subsequent two weeks and were treated with PDAT. Patient 1 received PDAT with 0.1% riboflavin and UV-A (365 nm) irradiation. Patient 2 received PDAT with 0.1% rose bengal and green (518 nm) irradiation. Irradiation energy was 5.4 J/cm^2 for both cases. After PDAT, a dry-preserved amniotic membrane and a bandage contact lens were placed. Topical antibiotics were continued throughout management.

Results: Patient 1: At presentation, slit-lamp exam revealed rapid progressive melting. Corneal thinning worsened causing an early descemetocele and PDAT was performed. One week after PDAT, the cornea had re-epithelialized and the ulcer shrank. After 6 months, the infection was cleared and an optical keratoplasty was performed.

Patient 2: At presentation, slit-lamp exam revealed severe corneal thinning and an inferior microperforation that was glued. With worsening clinical picture, patient received PDAT. Two weeks after PDAT, corneal melting halted, the cornea had re-epithelialized, and conjunctiva was quiet. After 7 months, the infection was cleared and an optical keratoplasty was performed.

Conclusions: PDAT successfully treated two patients with *Pseudomonas aeruginosa* keratitis in which medical therapy alone was not working. PDAT may be considered in the management of patients with infectious keratitis.

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Poster #37 Efficacy and Safety of SRG003 in Rhesus Monkeys (macaca mulatta) with Spontaneous Primary Optic Atrophy

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Purpose: SRG003 is an active ingredient of a medicinal herb with neuroregenerative properties. Synthetic SRG003 exhibits neuroprotective, neurite outgrowth and neurotrophic effects in neuronal models and positively impacts neurogenesis signaling pathways. Its effect was evaluated in two male Rhesus monkeys with spontaneous unilateral primary optic atrophy.

Methods: RNFL thickness were measured by OCT during a 4-month screening period followed by a 5-month treatment period. Neither monkey has diabetes, multiple sclerosis or glaucoma. They were treated orally with ascending doses of 5, 15, 30, 50, 75 mg/kg for a total of 5 months.

Results: RNFL thickness in both diseased eyes (OD) were stable during the screening period. SRG003 showed efficacy from 2-4 weeks. One monkey had a cumulative increase in RNFL thickness of 20-21 µm in nasal regions (N: 24 to 45 µm; NI: 85 to 105 µm); the other monkey had a cumulative increase of 14 µm (N: 15 to 29 µm; NS: 3 to 17 µm). No change was observed in both healthy eyes (OS). Both monkeys maintained normal body weight, food intake and daily activities. IOP, SL, or FP, blood routine or biochemistry did not change.

Conclusion: Increased RNFL thickness indicates an ability of SRG003 to reverse neurodegenerative processes in the retina. Decreased RNFL thickness in nasal subfields may predict cognitive decline (Ko et al, JAMA 2018). These *in vivo* results in monkeys converge with *in vitro* data to support a neuroregenerative potential for SRG003 and encourages its further research and development.

Poster #38 Ziv-aflibercept Efficacy in Better Regulating Neovascular AMD (ZEBRA) Trial

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Purpose: To determine if ziv-aflibercept is a safe, effective alternative to currently available anti-VEGF medications in eyes with neovascular age-related macular degeneration (nAMD).

Methods: This is a prospective, randomized, IRB-approved study. Inclusion criteria were active nAMD, prior treatment with aflibercept, ranibizumab, or bevacizumab, and BCVA≤20/250. Exclusion criteria included active intraocular inflammation, recent vitreous hemorrhage, and uncontrolled glaucoma. The treatment group received 1.25 mg/0.05mL intravitreal ziv-aflibercept, while the control group continued their existing anti-VEGF regimen. Primary outcomes were best corrected visual acuity (BCVA) and central foveal thickness (CFT).

Results: Of the 52 patients enrolled, 26 patients have been enrolled for at least nine months. Mean baseline BCVA in the control and treatment groups was 1.51±0.37 logMAR (Snellen equivalent: CF 6 ft) and 1.72±0.38 logMAR (Snellen equivalent: CF 5 ft) respectively, and mean change in BCVA was 0.17 logMAR and 0.07 logMAR respectively (p=0.45). Baseline CFT in the control and treatment groups was 246±62 µm and 240±95 µm respectively, and mean change in CFT was 40 µm and 21 µm respectively (p=0.49).

Ziv-aflibercept did not demonstrate any retinal toxicity, adverse events, vision loss, or significant change in mean arterial blood pressure during the study (p=0.35).

Conclusions: In this study, ziv-aflibercept was safe, well-tolerated, and effective in treating nAMD. Compared to aflibercept, ziv-aflibercept is non-inferior with respect to anatomy, function, and complication rate. Ziv-aflibercept may represent an important addition to current anti-VEGF treatment options, especially as a cost-effective alternative to aflibercept and as second-line therapy for eyes resistant to bevacizumab.

Poster #39 OKYO-0101, an agonist of G-protein coupled receptor (GPCR), ameliorates inflammation in an experimental model of dry eye disease in mice

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Purpose: The purpose of the study was to evaluate the ability of OKYO-0101, an agonist of Chemerin receptor, a member of GPCR family, to inhibit eye inflammation in a mouse model of scopolamine-induced dry eye.

Methods: Female C57Bl/6J mice were administered with subcutaneous injections of scopolamine (4 times a day for 5 Days) and housed in chambers with low humidity and constant airflow creating desiccating stress (DS) to induce acute dry eye disease (DED). Mice were randomized into 4 separate arms (n = 10) as follows. Arm 1: No treatment; Arm 2: Positive control; Arm 3: Vehicle and Arm 4: Test group. On Days 1-4, Arms 2-4 received bilateral topical administration (twice daily) of 0.1% Cyclosporine A (CsA-MiDROPS™), Vehicle, and OKYO-0101, respectively. Corneal permeability was assessed by Oregon Green Dextran (OGD) staining at baseline and at end of the studies. Eye tissues were collected and processed for histological quantification of conjunctival goblet cell (GC) density, and quantification of $CD4^+$ T-cells. Ocular tolerance is currently being evaluated in rabbits.

Results: DED-induced corneal permeability was reduced significantly by OKYO-0101 compared to vehicle group ($p \leq 0.001$). Potency of OKYO-0101 to reduce corneal permeably was comparable to cyclosporine, an active ingredient of Restasis® (Allergan). In addition, OKYO-0101 normalized DED-induced loss of GC density ($p \leq 0.001$) and reduced DED-induced enhancement of $CD4^+$ T-cells ($p \leq 0.05$), which are biomarkers of inflammation.

Conclusions: Topically instilled OKYO-0101 reduced symptoms of DED considerably in mice, suggesting its anti-inflammatory properties and it may **have** therapeutic potential for dry eye.

Poster #40 Extended Release of Protein to the Ocular Surface

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Purpose: To examine an extended release system that would expand treatment options for biomolecules in ophthalmology.

Methods: The formulations consisted of either 0.25% Insulin in PBS or 0.22% Insulin in PROLOC® gel. Fresh rabbit corneas or dialysis membranes were placed on spherical Franz Diffusion Cells. Solution cleared from the pre-corneal layer was analyzed via HPLC to evaluate drug retention and release profile for each formulation.

Results: The insulin solution formulation delivered a large amount of insulin that was quickly cleared from the ocular surface while the PROLOC® gel formulation delivered insulin at a sustained rate throughout the 5-hour study.

The PROLOC® formulation reduced the total amount of insulin cleared from the ocular surface compared to the solution formulation. PROLOC® gel was visible at the end of the study. Analysis confirmed insulin was still present in the gel on the cornea after 5 hours. No insulin remained in the cells dosed with solution.

Conclusions: The PROLOC® gel formulation continued to deliver drug at a steady rate throughout the 5-hour period studied while the solution formulation was rapidly removed from the ocular surface. PROLOC® has previously been shown to provide sustained release of an antibiotic from an ocular mini tablet. This study indicates in a gel form it is suitable for ocular delivery of a protein. Since PROLOC® can be supplied as a dry ocular mini tablet or as a partially hydrated gel it has the potential to provide both product stability and constant ocular drug delivery.

Poster #41 Lipid Metabolism Signaling and Phosphatase Inhibitors in Reducing Neuronal Apoptosis in Diabetic Retinopathy

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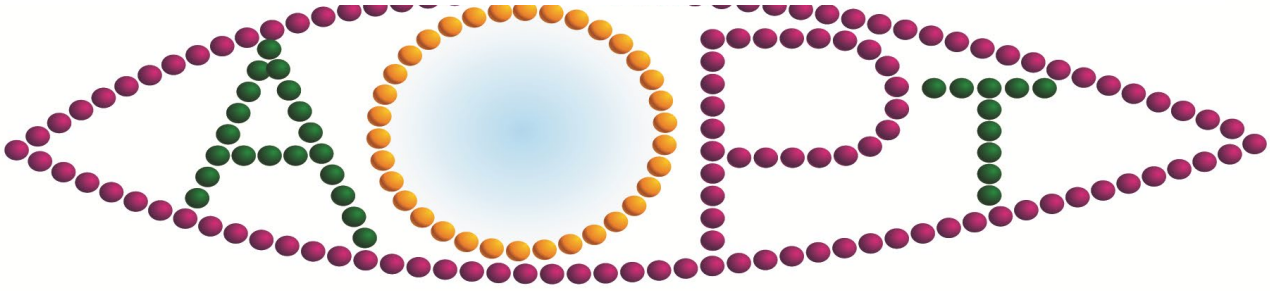
Purpose: Diabetic retinopathy (DR) is a visual complication of diabetes mellitus (DM). Retinal neurodegeneration precedes retinal vascular abnormalities used as indicators of DR. An important feature of retinal neurodegeneration is apoptosis of the retinal ganglion cells (RGCs). Previous studies with db/db mice (mimicking type 2 DM) indicate a lipid metabolism-regulated signaling pathway involving lipoprotein receptor-related protein 1 (LRP-1), suggesting that apoEdp (dimerized LRP-1-binding apolipoprotein E-derived protein) stimulates LRP-1 and triggers the PI3k/AKT pathway, lowering RGC apoptosis. Two phosphatases, protein phosphatase 2A (PP2A) and protein phosphatase 2B (PP2B), contribute to apoptosis antagonizing PI3k/AKT signaling. Current therapies have not addressed the key issues of early retinal neurodegeneration induced by hyperglycemia. We aim to determine the mechanisms of neuroprotection via the LRP-1 signaling pathway.

Methods: Intravitreal apoEdp treatment of diabetic db/db mice. Retinal protein extracts were used for western blot analysis. Retinas from 6-8-week-old neonatal C57BL/6 mice were used to prepare primary RGC cultures. ApopTag/TUNEL assays were used to quantify RGC apoptosis. Effects of varying concentrations of PP2A inhibitor endothall, PP2B inhibitor cyclosporine A, and apoEdp on RGCs were studied via immunofluorescence.

Results: Intravitreal apoEdp treatment induces (1) LRP-1 activation, (2) PP2A deactivation, (3) and reduces RGC loss in db/db mice retinas. *In vitro* results suggest that LRP-1 stimulation via apoEdp results in reduced glutamate-induced excitotoxicity and that cyclosporine A and endothall can reduce apoptosis of



Poster #42 Marijuana, Cannabinoids and Retina

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Purpose: There are currently ten states that have legalized recreational marijuana and thirty-three for medicinal purposes. There are increasing numbers using cannabis. We utilized Frequency Doubling Technology perimetry to assess the functional visual field and retinal function, of an opportunistically dosed group of non-chronic young adult marijuana users. Cannabinoids have been shown to enhance extreme peripheral vision, decrease glare recovery and impair perception of low spatial frequency contrast. Cannabinoid receptors have also been identified in multiple layers of the retina, the lateral geniculate nucleus, primary visual cortex, visual association cortex as well as other regions involved in vision processing. It is important to understand how cannabis affects these structures and possible resultant impairments.

Methods: Through a National Institutes of Health – National Institutes on Drug Abuse funded, Institutional Review Board approved protocol, we included visual screening at baseline and dosed sessions as part of a broader study related to impaired driving. Participants used their own marijuana without qualitative or quantitative dosing characteristics. However, saliva samples were taken during baseline and dosed testing to verify the presence or absence of marijuana and other drugs. Blood was also taken during dosed sessions to determine cannabinoids; 11-Hydroxy Delta-9 THC, Cannabidiol, Cannabigerol, Cannabinol; Delta-9 Carboxy THC and Delta-9 THC.

Results: Current trends reveal reduced function in the left superior quadrant, however this initial data is based on a small number of participants.

Conclusions: With more participants we predict we will be able to determine a retinal regional predilection for cannabinoids and potential brain lateralization.

Poster #43 Glucocorticoid-induced cell derived matrices modulate human trabecular meshwork cell behavior via integrins/caveolin-1-Rho GTPase axis

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Purpose: Trabecular meshwork (TM) extracellular matrix (ECM) remodeling is an important causal risk factor for ocular hypertension. However, partly due to inadequate mechanistic understanding and inappropriate ocular hypertensive models, limited therapeutics directly target the TM. Here, using glucocorticoid-induced cell derived matrices, we demonstrate how a remodeled matrix modulates TM cell behavior via integrins/caveolin-1-Rho GTPase axis.

Methods: Primary human TM (hTM) cells (n=5 donors) were cultured for 4 weeks in the absence/presence of dexamethasone to obtain vehicle control (VehM) and glucocorticoid-induced (GIM) matrices respectively. Subsequently, a fresh batch of hTM cells from the same donor was seeded on these matrices in growth media containing 1% FBS without further treatments for up to 7 days. Changes in protein expression and cellular biomechanics were quantified at 4 timepoints.

Results: Compared to hTM cells on VehM, αV and β5 integrins were overexpressed ($p<0.05$) in cells on GIM at all timepoints. While β1 integrin was increased ($p<0.05$) at a single timepoint (1d), β3 integrin was repressed at all timepoints. Phosphorylated focal adhesion and integrin linked kinases were upregulated at 5d. Caveolin-1 (Cav1) was upregulated at 1d, decreased at 3d, and overexpressed from 5d to 7d in cells on GIM. This trend was mostly mirrored by Rho GTPases, Rac1/2/3, Cdc42 and RhoA with Rac1/2/3 having more sustained effects. Concurrently, cells on GIM were stiffer ($p<0.05$) at all timepoints than VehM.

Conclusions: Specific integrins may serve as adhesion molecules in dysregulated cell-ECM interaction. However, close correlation between Cav1 and Rho GTPases, may implicate caveolae as critical mechanosensors.

Support: NIH T35 student Vision Research Support Grant (2018), Bright Focus National Glaucoma Research Award (VKR), and NIH/NEI grant 1 R01 EY026048-01A1 (VKR/JAV).

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43	Late Breaking (Travel Awardee)	Yemanyi	Glucocorticoid-induced cell derived matrices modulate human trabecular meshwork cell behavior via integrins/caveolin-1-Rho GTPase axis	153

AOPT-2019 Program Guide

March 7: Registration (15:00-17:15, Iberville Ballroom)

SESSION 1 (17:20 - 18:50, Queen Anne Ballroom) NOLA Ophthalmic Research (Moderators: Nicolas G. Bazan and Partha Bhattacharjee)

1. LRP-1 targeted retinal neuroprotection in diabetic db/db mice (Partha Bhattacharjee)
2. Antisense and Gene Therapy Rescues Hearing, Balance and Vision in Usher syndrome (Katelyn Robillard)
3. Molecular organization of lipids in the human macula and retinal periphery William Gordon)
4. Mechanisms by which ciliary neurotrophic factor (CNTF) protects rods and cones (Minghao Jin)
5. Neuroprotection by novel lipid mediators: significance in retinal degenerations(Nicolas G. Bazan)

Welcome Reception (18:50-20:00, Iberville Ballroom)

March 8: SESSION 2 (8:30 - 10:00, Queen Anne Ballroom) Therapeutic Modalities in Ophthalmology (Moderators: Ashwath Jayagopal and Dan Stamer)

1. AAV-mediated gene therapy for long-term effective intraocular pressure (IOP) control in a canine open-angle glaucoma (OAG) model (Andras Komaromy)
2. Bimatoprost Sustained-Release Implants for Glaucoma Therapy (Mike Robinson)
3. Activation of PPARa, a Potential therapeutic strategy for Age-Related Macular Degeneration (Jian-xing Ma)
4. Development of Luxturna™ (voretigene neparvovec-rzyl): Gene Therapy for RPE65 Biallelic Mutation Associated Inherited Retinal Disease (Dan Chung)
5. Small molecule ligand targeting of locked nucleic acids to enable corneal delivery (Kameron V. Kilchrist)

Break & Exhibits (10:00-10:20)

SESSION 3 (10:20 - 11:50) Inflammation in Retinal Degenerative Diseases: Immune Therapy (Moderators: Heping Xu and Florian Sennlaub)

1. HTRA1 inactivates thrombospondin-1 mediated subretinal immune-suppression (Florian Sennlaub)
2. An immune target for neuroprotection in glaucoma (Dongfeng Chen)
3. Role and regulation of the innate inflammatory system in diabetic retinopathy (Patrice E. Fort)
4. Monocyte-Derived Macrophages in Diabetic Retinopathy (Xavier Guillonneau)
5. Immune suppression as an alternative approach to control retinal angiogenesis (Heping Xu)

LUNCH SYMPOSIUM (11:50 - 13:00, March 8, Orleans A room and room TBD) Maximizing collaboration between academia and industry (Panelists academia: Tom Yorio, Carol Toris, Iok-Hou Pang, Achim Krauss, Ashwath Jayagopal)

SESSION 4 (13:00 - 14:30) Gene Therapy Approaches in Treating Eye Diseases (Moderators: Alfred Lewin, Stephen Tsang and Jijing Pang)

1. Precision Genome Surgery for Imprecision Medicine(Stephen Tsang)
2. Knocking down blindness: a gene therapy for autosomal dominant retinitis pigmentosa (William A. Beltran)
3. Targeting the PERK arm of the Unfolded Protein Response in Retinal Degeneration (Marina Gorbatyuk)
4. Gene therapy for Leber's Hereditary Optic Neuropathy (Jiajia Yuan)

Break & Exhibits (14:30-14:50)

SESSION 5 (14:50 - 16:20) YOUNG INVESTIGATOR (TRAVEL AWARDEE) SESSION (Moderators: Malinda Fitzgerald and Monica Jablonski)

1. AAV delivery of modified erythropoietin (EPO) therapy delays retinal degeneration in a mouse model of geographic atrophy (Manas Biswal)
2. Degradable fibrin scaffolds for induced pluripotent stem cell (iPSC)-retinal pigment epithelium (RPE) Transplantation Using a Pig Model (Jarel Gandhi)
3. Pregabalin Microemulsion Once Daily Eye Drops for Management of Glaucoma (Mohamed M Ibrahim)
4. Vasoregulators mediate distal vessel lumen diameters and outflow facility in human anterior segments (Fiona McDonnell)
5. A CRISPR-based inducible system for VEGF repression for AMD (Bo Yu)
6. TRPV4 -dependent calcium influx regulates strain-induced neurodegenerative pathways in retinal ganglion cells (Monika Lakk)

AOPT GENERAL BUSINESS MEETING (16:20 - 17:20, Queen Anne Ballroom) for AOPT members

POSTER SESSION (17:30 - 19:00, Iberville Ballroom) (Moderators: Maria Reinoso and Juana Gallar)

March 9: SESSION 6 (8:30 - 10:00) New Approaches for Treating Age-related Macular Degeneration (AMD) (Moderators: Cathy Bowes Rickman and James Handa)

1. The Role of Mitochondrial Dysfunction in the Pathogenesis of Dry Age-Related Macular Degeneration: From Concept to Clinic for the Mitochondria-directed Drug, Elamipretide (Scott Cousins)
2. TFEB (transcription factor, EB) as a potential therapeutic target for AMD (Debasish Sinha)
3. The Conundrum of Targeting the Complement Pathway to Treat AMD – Lessons from Animal Models (Catherine Bowes Rickman)
4. A roadmap to find treatment for dry AMD (James Handa)
5. Targeting soft drusen in age-related macular degeneration (AMD): rationale and pre-clinical studies of an apolipoprotein mimetic peptide (Christine Curcio)

Break & Exhibits (10:00-10:20)

SESSION 7 (10:20 - 11:50 March 9) Latest Development in Neuroprotection for Glaucoma (Moderators: Raghu Krishnamoorthy and Rebecca Sappington)

1. Synaptic disassembly and rewiring of the adult retina in a mouse model of glaucoma (Luca Della-Santina)
2. Higher Reliance on Glycolysis Limits Responsiveness in Degenerating Glaucomatous Optic Nerve (Denise Inman)
3. Interleukin-6 in Retinal Health and Disease(Rebecca Sappington)
4. Microvascular dysfunction and role of pericytes in glaucoma (Luis Alarcon-Martinez)
5. Alpha B crystallins in glaucoma neuroprotection (Dorota Stankowska)
6. A simple chronic ocular hypertensive murine model of glaucoma – opportunities for neuroprotection studies (Chenying Guo)

LUNCH-N-LEARN ROUNDTABLE (11:50 - 13:00) Orleans A room and room TBD How to setup and run a new lab in academic setting? (Panel Leaders: Carol Toris, Christine Wildsoet and Vivian Lee)

SESSION 8 (13:00 - 14:30) Drug Discovery and Development of Novel Ocular Therapeutics (Moderators: Carol Toris and Iok-Hou Pang)

1. Clusterin regulates intraocular pressure by modulating extracellular matrix in trabecular meshwork outflow pathway (Padmanabhan Pattabiraman)
2. Ligandomics for retinal angiogenesis drug discovery (Wei Li)
3. Metabolomics for ocular drug discovery (Sanjoy Bhattacharya)
4. Epigenetic Modulators as Novel Therapeutics: Translational Perspectives on 20 Years of Preclinical Success (Jeffery Gidday)
5. 21st Century Ocular Pharmacology and Therapeutics: Viral Vectors as Drugs (Carl Romano)
6. Lessons learned from drugs that fail (Stephen Poor)

Break & Exhibits (14:30-14:50)

SESSION 9 (14:50 - 16:20) Advances in Ophthalmic Drug Delivery (Moderators: Chris Wildsoet and David Waterbury)

1. Specific drug targeting to enhance treatment efficacy (Ilva Rupenthal, PhD)
2. Can Drug Delivery Enhance the Efficacy of Ocular Therapeutics (Heather Sheardown)
3. Suprachoroidal drug delivery to the eye (Uday B. Kompella)
4. Novel topical formulation for glaucoma (Monica Jablonski)
5. A new nanomedicine method for treating corneal graft rejection (Qingguo Xu)

KEYNOTE ADDRESS (17:00 - 18:20, Queen Anne Ballroom) (Moderator: Thomas Yorio) BETWEEN A ROCK AND A HARDENED MESHWORK: THE DISCOVERY AND DEVELOPMENT OF RHOPRESSA (Casey Kopczynski)

Banquet Dinner (19:00-21:30), Royal Ball Room

March 10: SESSION 10 (8:30 - 10:00) Novel Therapies for Corneal Diseases (Moderators: Claudio Bucolo and Filippo Drago)

1. Novel lipid mediators and neurotrophins targeting cornea nerve integrity (Haydee Bazan)
2. Cell therapy and gene therapy in eye diseases (Graziella Pellegrini)
3. Old and new cation channel blockers to treat ocular discomfort and pain (Juana Gallar)
4. Neuropathic corneal pain: approaches for management (Pedram Hamrah)
5. Xanthohumol protects corneal epithelial cells against oxidative stress in vitro (Simon Kaja)
6. The miR-29b Mimic Remlarsen as an Anti-Fibrotic Therapeutic in the Eye (Corrie, Gallant-Behm)

Break & Exhibits (10:00-10:20)

SESSION 11 (10:20 - 11:50) Hot Topics from Abstract Submission (Moderators: Ze Zhang and Rajashekhar Gangaraju)

1. Exploration of the secretome of adipose stem cells for the design of retinal therapeutics (Rajashekhar Gangaraju)
2. Novel topically delivered small molecule with IOP lowering and neuroprotective activity (Suchismita Acharya)
3. Failure of Oxysterols Such as Lanosterol to Restore Lens Clarity from Cataracts (Peter Kador)
4. More than just a reactive oxygen species scavenger: grapes prevent UV-B radiation-induced cataract by upregulating anti-apoptotic protein XIAP (HongLi Catherine Wu)
5. The Endothelin Receptor Antagonist Macitentan Attenuates Neurodegeneration in a Rodent Model of Glaucoma and Ameliorates Endothelin-Mediated Vasoconstriction (Raghu Krishnamoorthy)
6. Protection of kaempferol on oxidative stress-induced retinal pigment epithelial cell damage (Donglei Zhang)

AOPT Board Meeting (12:00-13:00, Bienville C)

SESSION 12 (13:00 - 14:30) Disruptive Technologies: Ophthalmic Tools and Methods that Have Changed the Ways We See the Eye (Moderators: Cheryl Rowe-Rendleman and Randolph Glickman)

1. Audacious Goals Initiative: Status and Impact (Steven Becker)
2. Photoacoustic Imaging and Sensing: a New Way to See the Eye (Randolph Glickman)
3. A Platform to Take on the Entire Progressive Retinal Degeneration Disease Continuum (Francois Binette)
4. Early stage detection of Glaucoma by monitoring nanostructure and function of RGC layer using Multifractal OCT (Subrata Batabyal)
5. Preclinical Evaluation of ADVM-022, a Novel Gene Therapy Approach to Treating Wet Age-Related Macular Degeneration (Claire M. Gelfman)

Panel Discussion (moderated by Cheryl Rowe-Rendleman)

Break & Exhibits (14:30-14:50)

SESSION 13 (14:50 - 16:20)What Every Ophthalmologist Needs to Know About the FDA (Moderators: (Bing Cai and Jayne Weiss)

1. Advancing Technology Challenges in Ophthalmic Drug Approvals (Wiley A Chambers)
2. Generic Drugs and Their Role in Bringing Next Generation Products: An FDA Perspective (Markham Luke)
3. Generic Ophthalmic Drug Products, Physical Characteristics and Bioequivalence (Darby Kozak)
4. How FDA Ensures Quality of Ophthalmic Drug Products (Patricia Onyimba)