Intraocular Pressure / Aqueous humour dynamics

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Purpose: Bradykinin (BK), a nonapeptide has been shown to regulate IOP in different species including rabbits and monkeys. Presently we intended to characterize the IOP lowering effects of BK2A78, a novel selective non-peptide bradykinin B2 receptor agonist in ocular hypertensive (OHT) cynomolgus monkeys.

Methods: BK2A78 was evaluated in several in vitro efficacy and receptor binding assays using human cloned B1 and B2 CHO cells and in human ciliary smooth muscle (HCM) cells. Intracellular calcium mobilization ([Ca2+]i) was assessed using FLIPR assay and prostaglandin (PG) release was measured using an EIA assay. Ocular safety assessments including slit lamp examinations were performed in monkeys following topical ocular application of BK2A78. IOP changes were measured using an Alcon computerized pneumotonometer in normal and hypertensive eyes of cynomolgus monkeys.

Results: BK2A78 is a selective B2 receptor agonist with EC50 values of 13 ± 5 nM (Emax = 92 ± 1%; BK response was 100%) in [Ca2+]i assay and 12 ± 7 nM (Emax = 100 ± 14%) in the PG release assays respectively. BK2A78 exhibited comparable high affinity binding to B2 receptors (Ki = 3 – 10 nM) with no detectable affinity towards B1 receptors. Topical ocular dosing of BK2A78 (3 ug x 3 times, 1 hour apart) to sedated cynomolgus monkeys caused no flare or cells to appear in the anterior chamber as observed up to 24h post-dose. No other adverse effects were noted. A single topical ocular application of BK2A78 (0.03 – 3 ug) caused a dose-dependent IOP reduction up to 25% from baseline between 6 – 24h post-dose in hypertensive eyes of conscious cynomolgus monkeys. Maximal percent IOP reduction of 25% was observed at 0.9 – 3 ug doses. The duration of action appeared to last >24h for BK2A78.

Conclusions: Bradykinin B2 receptor agonism appears to cause IOP lowering. Unlike the issues surrounding topical ocular application of BK peptide, including non-selectivity against B1 and B2 receptors, poor ocular penetration and susceptibility to rapid degradation by angiotensin converting enzyme, BK2A78 offers several new therapeutic advantages. BK2A78 is a selective non-peptide B2 receptor agonist capable of robust and long lasting IOP reduction that appears to also be well tolerated following topical ocular dosing in OHT monkeys.

Commercial Relationships: Ganesh Prasanna, Novartis Inst Biomedical Research (E); Naj Sharif, Alcon Research Ltd (E); Byron H. Li, NIBR (E); Mark Hellberg, NIBR (E); Terri Krause, NIBR (E); Shenouda Yacoub, NIBR (E); Daniel Scott, NIBR (E); Curtis R. Kelly, NIBR (E); Iok-Hou Pang, None; Keith Combrink, None; Mark Hellberg, Biomedical Research (E);

The Use of Tissue Plasminogen Activator to Reduce Elevated Intraocular Pressure Induced by Prednisolone in Sheep

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Purpose: We have previously shown (ARVO 2013) that tissue plasminogen activator (tPA) injected into the vitreous of sheep reduced or prevented the elevation of the intraocular pressure (IOP) normally produced by the instillation of 1% prednisolone.

We present these data in order to show that IOP elevation may be the result of an effect on extra-cellular matrix turnover in the TM. These findings suggest that tPA may be useful as a therapeutic agent in steroid-induced glaucoma.

Commercial Relationships: Oscar A. Candia, None; Rosana Gerometta, None; John Danias, None

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Simulating the effect of trabecular meshwork resistance and episcleral venous pressure on conventional aqueous humor outflow dynamics

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Purpose: To simulate the effect of trabecular meshwork (TM) resistance and episcleral venous pressure (EVP) on conventional outflow dynamics in an artificial model.

Methods: We constructed an artificial perfusion model comprising a microsyringe on a pump (representing aqueous inflow) connected to needles of different caliber (33G and 35G; TM resistor) linked to a one-way valve (Schlemm’s canal inner wall endothelium barrier) and then a static fluid column (EVP). Three intervening pressure transducers (PT) were used: PT#1: between pump and needle (representing intraocular pressure (IOP)); PT#2: between needle and...
Mechanical and cytokine stimulation of smooth muscle actin expression in human trabecular meshwork cells

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Purpose: The goal of the present study was to determine the impact of mechanical stress and transforming growth factor beta-2 (TGF-β2) on the protein expression of the smooth muscle actin (SMA) in human trabecular meshwork (TM) cells.

Methods: Primary cultures of human TM cells isolated from three different donor eye pairs were used as models. Confluent and mature monolayers of TM cells were subjected to either (i) TGF-β2 (0.1-5 ng/ml) for 24 hr or (ii) TGF-β2 (0.1-5 ng/ml) for 6, 24 and 48 hours or (iii) cyclic mechanical stretch (16% strain at 1 Hertz) in a Flexcell System ±TGF-β2 (5ng/ml) for 24 hours. Relative expression of SMA was determined by Western Blot using anti-SMA specific IgGs normalized to total protein on nitrocellulose blots by Pierce reversible stain and imaged analysis.

Results: SMA protein levels in human TM cells increased 3.5 fold (p=0.03) after 5ng/ml TGF-β2 treatment, but remained unchanged at lower concentrations (0.1 and 1ng/ml). Maximum effects of TGF-β2 were observed at 48 hours after treatment. By comparison, exposing human TM cells to mechanical stretch increased SMA 2-fold at 6 hours and 3-fold at 24 hours (p<0.05). The combination of mechanical stretch and TGF-β2 treatment synergistically increased SMA production after 24 hours. (n=2)

Conclusions: Results show that human TM cells rapidly upregulate their contractile machinery in response to both mechanical and cytokine stimuli, and when present together responses are exacerbated. Prolonged stimulation may facilitate transdifferentiation of TM cells to accommodate stress in a homeostatic or pathological fashion, such as may occur in glaucoma.
Role of Caveolin-1 in Intraocular Pressure and Conventional Outflow Regulation

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**Purpose:** Gene association studies have linked polymorphisms in the CAV1/2 gene locus to risk of primary open angle glaucoma. The proteins encoded by these genes are the signature structural proteins of caveolae plasma membrane domains that are abundant in the conventional outflow pathway including Schlemm’s canal endothelium and trabecular meshwork. The purpose of this study was to test consequences of caveolin-1 (Cav-1) ablation on intraocular pressure (IOP), conventional outflow, and outflow pathway morphology.

**Methods:** Global Cav-1 knockout (KO) mice and age/sex-matched controls were used for these studies. Intraocular pressure was measured by rebound tonometry (Tonolab), outflow facility was measured in perfused enucleated eyes using a constant flow perfusion system customized for mice, and outflow tissue morphology was assessed by light and transmission electron microscopy.

**Results:** A significant elevation in IOP was observed in Cav-1 KO mice at 5 weeks of age and was sustained at 3 and 6 months. Pressure-dependent outflow, measured at 4 sequentially increasing pressure steps (8, 15, 25, 35 mmHg), was significantly reduced by 43% in Cav-1 KO mice compared to controls (0.045 ± 0.012 versus 0.079 ± 0.005 µl/min/mmHg; p ≤ 0.01 versus control eyes). Ultrastructural analysis revealed a loss of morphologically-identifiable caveolae. Schlemm’s canal endothelial cells were considerably thicker and shorter than in controls, and protruded into the lumen of the canal. Typical giant vacuoles were absent.

**Conclusions:** Results provide compelling evidence that Cav-1 and caveolae play important roles in conventional outflow and thus IOP regulation. Understanding the mechanism by which Cav-1 controls pressure-dependent outflow has important clinical implications for both the pathobiology and treatment of glaucoma.

**Commercial Relationships:** Michael H. Elliott, None; Xiaowu Gu, None; Nicole E. Ashpole, None; Gina L. Griffith, None; Timothy M. Boyce, None; Masaki Tanito, None; Ernst R. Tamm, None; W Daniel Stamer, None

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### OD and OS IOPS vs Date of Administration (Times of doses and Concentration of doses)

**Commercial Relationships:** Jonathan B. Jacobs, None; Richard W. Hertle, US FDA (P), Vision of Children (F); Jeffrey Dunmire, None; Louis F. Dell’Osso, None; Lauren A. Dalvin, None; Dongsheng Yang, None; Michelle Evano-Chapman, None

**Support:** Akron Children

**Program Number:** 2889 Poster Board Number: B0287

**Presentation Time:** 8:30 AM–10:15 AM

**Efficacy and Safety of Topical New Sodium Pump Inhibitor (NSPI) in Reducing Intraocular Pressure in a Canine Model**

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**Purpose:** To determine the ocular and systemic safety and efficacy of a new topical medication in canines to lower intraocular pressure. This additional effect was discovered during a study of an NSPI as a treatment for infantile nystagmus syndrome.

**Methods:** Following in vitro safety testing and IUCAC approval, we applied increasing concentrations of topical NSPI drops (0.002% to 0.7%) in two canines with normal intraocular pressures. Drops were applied routinely in both animals. We collected ophthalmic data including: rebound tonometry (ICARER), indirect ophthalmoscopic video fundus examination, and slit lamp and external inspection for surface and intraocular toxicity. We monitored for systemic toxicity with urine analysis and venous blood sampling for hematology, serum chemistries and liver function tests.

**Results:** At the 0.7% concentration dose of NSPI there was minimal, reversible, conjunctival hyperemia, with no other ocular or systemic toxicity. At the 0.6% dose there was a sustained decrease in IOP of 40-60%. Pressure returned to—or near—normal by the following day, though after prolonged administration the rebound lessened.

**Conclusions:** Canines serve as an excellent model for human glaucoma. They share similar IOP, anterior segment anatomy and aqueous production, metabolism and egress physiology. We postulate an immediate decrease in aqueous production as the likely mechanism of the NSPI. This unique class of biologically acting agents may act directly on non-pigmented ciliary epithelial metabolism. Our study suggests that a new, potent, safe class of pharmacological agents has potential for topical treatment of human glaucoma.

**Program Number:** 2889 Poster Board Number: B0288

**Presentation Time:** 8:30 AM–10:15 AM

**EFFECT OF HYDROGEN SULFIDE-RELEASEING COMPOUNDS ON AQUEOUS HUMOR DYNAMICS**

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**Purpose:** The presence of biosynthetic enzymes for hydrogen sulfide (H$_2$S) production coupled with reports of its pharmacological action in ocular tissues, suggests a potential role for this novel gasotransmitter in intraocular pressure (IOP) regulation. In this study, we investigated the effects of H$_2$S (generated from donor, sodium hydrosulfide (NaHS), and substrate, L-cysteine) on IOP and aqueous humor (AH) outflow facility.

**Methods:** A single dose of H$_2$S-releasing compounds, NaHS and L-cysteine (vehicle: normal saline) was topically applied to each eye of New Zealand Albino rabbits. IOP was measured with a pneumotonometer at baseline and at regular intervals (30 - 120 minutes) until baseline IOP was stable. For AH outflow studies, porcine ocular anterior segment explants with intact trabecular meshwork were perfused with DMEM maintained at 37°C, 5% CO$_2$ and constant pressure of 7.35 mmHg. Stabilized explants were exposed to NaHS and L-cysteine. Explants were also treated with the K$_{ATP}$ channel antagonist glibenclamide and H$_2$S biosynthetic enzyme inhibitors, aminooxyacetic acid (AOA), or proparglyglycine (PAG).

**Results:** NaHS (1%) elicited a significant time-dependent ($p < 0.001$) reduction in IOP in treated eyes that reached a maximum at 3 hours and remained sustained for 6 hours. L-cysteine (5%) also reduced IOP for up to 7 hours, achieving a maximal IOP reduction of 28.8% ($p<0.01$) after 3 hours. Moreover, both compounds elicited a parallel but smaller reduction of IOP in contralateral, vehicle-treated eyes. Interestingly, L-cysteine (1 nM - 1 μM) caused a dose-dependent increase in AH outflow from porcine anterior segment explants, reaching a maximal effect at 100 nM [153 ± 7.2% of basal (mean ± SE)]. The effect of L-cysteine (100 nM) on AH outflow was completely attenuated by AOA (30 μM) and PAG (1 μM). NaHS (100 nM – 10 μM) also produced a concentration-dependent increase in AH outflow, reaching a maximal effect at 10 μM. In addition, the enhancement of outflow caused by both NaHS (10 μM) and L-cysteine (100 nM) was inhibited significantly ($p < 0.01$) by glibenclamide (100 μM).

**Conclusions:** We conclude that compounds that produce H$_2$S can lower IOP in normotensive rabbits. In porcine eyes, the H$_2$S-induced decrease in IOP is due to an increase in AH outflow via the trabecular meshwork. Furthermore, the H$_2$S-induced increase in AH outflow is dependent upon the intramural biosynthesis of H$_2$S and, is mediated by K$_{ATP}$ channels.

**Commercial Relationships:** Ya Fatou Njie-Mbye, None; Jenaye Robinson, None; Chinonso Ezeudu, None; Leah Mitchell, None; Madhura Kulkarni-Chitnis, None; Catherine A. Opere, None; Sunny E. Ohia, None

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**Program Number:** 2891 Poster Board Number: B0289
**Presentation Time:** 8:30 AM – 10:15 AM

**Ocular Hypotensive Effect of Baicalein in Sprague-Dawley Rats**

Hoi-Lam Li, Chi-Ting Leung, Ho-Lung Chan, Chi-ho To, Chi-wai Do.

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**Purpose:** Baicalein (5,6,7-trihydroxyflavone) is a natural flavonoid (flavone) originally derived from the root of Scutellaria baicalensis. We have previously demonstrated that baicalein suppresses the net CI- transport and fluid movement across the excised ciliary epithelium, potentially reducing the aqueous humor formation and intraocular pressure (IOP). The inhibitory effect of baicalein is possibly mediated by the inhibition of swelling-activated CI- channels in non-pigmented ciliary epithelial cells. In this study, we aim to evaluate the IOP-lowering ability of baicalein in Sprague-Dawley (SD) rats.

**Methods:** Adult 2-4 months old male SD rats were used. Intraperitoneal injection of baicalein was administered once daily for a period of 4 weeks. In the treatment group, baicalein was applied at a concentration of 4mg/kg in the first 2 weeks followed by a higher concentration of 40mg/kg in the last 2 weeks. Saline solution was used in the control group throughout the experiment. The measurements of IOP were conducted in both eyes under anesthesia by a rebound tonometer.

**Results:** Intraperitoneal injection of baicalein significantly lowered baseline IOP in SD rats in vivo. After treatment with baicalein at a concentration of 4mg/kg, baicalein-treated SD rats have a lower IOP of $1.2±1.0mmHg$ as compared to the saline-treated controls (N=8; P<0.05). Subsequently, at a higher concentration of baicalein (40mg/kg), the hypotensive effect was progressively increased to 2.7±1.3mmHg after 4-week of treatment (N=8; P<0.01). The ocular hypotensive effects of baicalein at concentrations of 4mg/kg and 40mg/kg were 9% and 20%, respectively, over the baseline IOP.

**Conclusions:** Baicalein seems to be a good candidate for glaucoma therapy in view of its ocular hypotensive effect. Whether or not these findings can be applied in human subjects are yet to be established and validated.

**Commercial Relationships:** Hoi-Lam Li, None; Chi-Ting Leung, None; Ho-Lung Chan, None; Chi-ho To, None; Chi-wai Do, None
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**Program Number:** 2892 Poster Board Number: B0290
**Presentation Time:** 8:30 AM – 10:15 AM

**Effects of Brimonidine on the Intraocular Pressure and Blood-Aqueous Barrier Permeability after Phacoemulsification and Intraocular Lens Implantation in Glaucoma Subjects**


**Purpose:** Preoperative and immediate postoperative treatment with an alpha 2 stimulant, apraclonidine, reduces the early post-surgical increases in the blood-aqueous barrier permeability and intraocular pressure (IOP) in eyes after successful cataract surgery. We herein evaluated the effects of another topical alpha 2 stimulant, brimonidine, on the early postoperative IOP and blood-aqueous barrier permeability in subject with glaucoma after cataract surgery.

**Methods:** We conducted a prospective randomized clinical trial in which 37 patients undergoing uncomplicated phacoemulsification and posterior chamber intraocular lens implantation were randomly assigned to treatment with 0.1% brimonidine or control. One drop of 0.1% brimonidine was instilled 1/2 hour preoperatively and immediately postoperatively. The IOP and aqueous flare intensity were determined before the operation and six hours, 24 hours and seven days after the operation using a Goldmann applanation tonometer and a laser flare-cell meter. The IOP just after surgery was adjusted between 10-15 mmHg using a Tono-Pen with sterilized tonometer and a laser flare-cell meter. The IOP just after surgery was adjusted between 10-15 mmHg using a Tono-Pen with sterilized tonometer. Anti-glaucoma eye drops were used without cessation. All procedures adhered to the tenets of the Declaration of Helsinki, and their use was approved by the Institutional Review Board of Hiroshima University, Japan.

**Results:** Eighteen eyes were assigned to the brimonidine group and 19 eyes to the control group. An IOP increase was observed six hours postoperatively in both groups. The IOP in the control group (19.2±4.0 mmHg) was significantly higher than that in the brimonidine group (15.8±4.9 mmHg, P=0.034). The aqueous flare intensity was highest six hours after surgery. There were no significant differences between the two groups.

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Conclusions: Although the topical application of brimonidine before and after cataract surgery successfully prevented the IOP elevation in glaucoma subjects, brimonidine could not suppress the flare intensity increase.

Commercial Relationships: Yoshiaki Kiuchi, None; Kaori Ideguchi, None; Yousuke Sugimoto, None; Taichiro Chikama, None

Clinical Trial: UMIN000007152

Program Number: 2893 Poster Board Number: B0291
Presentation Time: 8:30 AM–10:15 AM
Effect of Pilocarpine on Intraocular Pressure and Schlemm’s Canal in Experimental Glaucomatous Monkey Eyes

Shenouda Yacoub, Byron H. Li, Rad Daly, Sarah S. Webb, Brian Thomas, Glen Jernigan, Quinn Sessums, Daniel Scott, Ganesh Prasanna, Dennis S. Rice. Glaucoma Research, Novartis Institutes for Biomedical Research, Fort Worth, TX.

Purpose: To assess the usefulness of enhanced depth imaging optical coherence tomography (EDI OCT) for evaluating the in vivo microarchitectural features of the trabecular-Schlemm’s canal (SC) outflow pathway.

Methods: Chronic ocular hypertension (OHT) was induced in the right eye (OD) of 9 female, 4 year old, cynomolgus monkeys by argon laser trabecular photococagulation. The left eye (OS) was untreated with normal intraocular pressure (IOP). The effect of 300 μg Pilocarpine on IOP in eyes of conscious animals was measured with a pneumotonometer following a single topical ocular instillation. In a separate study, the effect of same dose Pilocarpine on SC was in vivo imaged in the same group animals under gas anesthesia. A serial of 49 horizontal EDI OCT B-scans in a 15x3 degree rectangle area were obtained by Spectralis SD-OCT (Heidelberg Engineering, Germany) in the temporal limbal area each eye. SC was visualized as a dark non-reflective area in the cross-sectional image of each B-scan, and was measured by three independent masked observers using AMIRA V. 5.4.5 (FEI Visualization Sciences Group, Burlington, MA). The parameters measured before and post dose were then processed for comparative analyses.

Results: In the IOP study, a single dose of 300 μg Pilocarpine induced, at 1 hour post-dose, the maximum and significant IOP reduction (7.6 mmHg or 21.5±2.9%) from baseline in the lasered OHT eyes and (3.5 mmHg or 13.9±2.7%) in the fellow normal eyes, respectively. In the imaging study, the mean SC area was significantly increased from 1,563±590 μm² to 2,568±620 μm² (64%) in the lasered OHT eyes and from 1,784±448 μm² to 3,218±449 μm² (80%) in the normal fellow eyes 1 h post dose, respectively.

Conclusions: EDI OCT is useful for evaluating the in vivo microarchitecture of the trabecular-SC outflow pathway. This technology has important implications for the development of new drugs and techniques to reduce intraocular pressure.

Commercial Relationships: Shenouda Yacoub, Novartis Institutes for Biomedical Research (E); Byron H. Li, Novartis Institutes for Biomedical Research (E); Rad Daly, Novartis Institutes for Biomedical Research (E); Sarah S. Webb, Novartis Institutes for Biomedical Research (E); Brian Thomas, Novartis Institutes for Biomedical Research (E); Glen Jernigan, Novartis Institutes for Biomedical Research (E); Quinn Sessums, Novartis Institutes for Biomedical Research (E); Daniel Scott, Novartis Institutes for Biomedical Research (E); Ganesh Prasanna, Novartis Institutes for Biomedical Research (E); Dennis S. Rice, Novartis Institutes for Biomedical Research (E)

Program Number: 2894 Poster Board Number: B0292
Presentation Time: 8:30 AM–10:15 AM
Effect on physiologic intraocular pressure of four topic formulations of valsartan and its impact on conjunctival irritation: a pilot study in rabbits


Purpose: To examine and compare the intraocular pressure (IOP) lowering effect and conjunctival irritation (hyperemia, chemosis and conjunctival discharge) of four different topical valsartan formulations (1.0%, 2.0%, 3.0% and 4.0%) in healthy rabbits.

Methods: All experiments were conducted in accordance with the Association of Research in Vision and Ophthalmology’s (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. The research was approved by an internal ethics committee for animal experimentation. Twelve healthy New Zealand White rabbits were treated with either, valsartan 1.0%, valsartan 2.0%, valsartan 3.0% or valsartan 4.0% (3 animals/group). One dose of study article was administered to both eyes of each animal every 12 hours for 5 days. IOP was assessed on days 2 and 5 using a Goldman tonometer and conjunctival irritation was assessed using a slit-lamp biomicroscope to evaluate hyperemia, chemosis and conjunctival discharge.

Results: The IOP mean decrease on the second day was 2.1mmHg, 2.3mmHg, 1.8mmHg and 2mmHg for the valsartan 1.0%, 2.0%, 3.0% and 4.0% respectively, on the fifth day the decrease compared to baseline was 2.1mmHg, 2.5mmHg, 2mmHg and 2.6mmHg respectively. Comparing the percentage reduction of baseline versus final IOP, no statistical differences were found in the inter-group comparisons. No chemosis or conjunctival discharge were found in any study group. There was no statistical difference between groups for the conjunctival hyperemia.

Conclusions: The four formulations of valsartan have lowering pressure properties, despite the higher 4.0% drug concentration, valsartan 4.0% does not increase the conjunctival irritation. Further studies would be needed to determine the long term effect on IOP and safety of valsartan formulations.

Commercial Relationships: Oscar Olvera Montaño, Laboratorios Sophia S. A. de C. V. (E); Yusset Contreras Rubio, Laboratorios Sophia S. A. de C. V. (E); Leopoldo M. Baiza-Duran, Laboratorios Sophia S. A. de C. V. (E); Paloma A. Márquez, Laboratorios Sophia S. A. de C. V. (E)

Program Number: 2895 Poster Board Number: B0293
Presentation Time: 8:30 AM–10:15 AM
In Vivo Dimensions of Schlemm’s Canal in Normal and Experimental Glaucomatous Monkey Eyes

Byron H. Li, Shenouda Yacoub, Rad Daly, Sarah S. Webb, Brian Thomas, Quinn Sessums, Glen Jernigan, Terri Krause, Ganesh Prasanna, Dennis S. Rice. Glaucoma Research, Novartis Institutes for Biomedical Research, Fort Worth, TX.

Purpose: To characterize in vivo structure of the Schlemm’s canal (SC) in normal and experimental glaucomatous monkey eyes.

Methods: Chronic ocular hypertension (OHT) of various durations (2.5-11 y) was induced in the right eye (OD) of 17 cynomolgus monkeys by argon laser trabecular photocoagulation. The left eye (OS) was untreated with normal intraocular pressure (IOP). An additional 15 native animals served as controls. A serial of 49 horizontal enhanced depth imaging (EDI) optical coherence tomography (OCT) B-scans in a 15x3 degree rectangle area were obtained in gas anesthetized monkeys by Spectralis SD-OCT (Heidelberg Engineering, Germany) in the temporal limbal area.
each eye. A dark non-reflective area in the cross-sectional image of each EDI OCT B-scan is considered as the SC and was measured by three independent masked observers using AMIRA V. 5.5 (FEI Visualization Sciences Group, Burlington, MA). The mean SC area of the 49 scans was then calculated.

**Results:** In the 15 naive male monkeys, the mean cross-sectional SC area in OD and OS was 3,322 ± 367 µm² and 3,541 ± 451 µm², respectively and without significant difference. In three age-matched male experimental monkeys (1 year post laser treatment), the mean SC area in the lasered eyes (1035 ± 323 µm²) was significantly smaller than that in the non-lasered fellow eyes (4196 ± 387 µm²). On the contrary, the mean SC area in the normal eyes of the 9 age-matched female monkeys was only 1784 ± 448 µm² (about half of that in male monkeys) and the SC area in the lasered eyes became slight narrower 1563 ± 590 µm² but without significant difference from the fellow eyes. In the 5 older monkeys who have longer duration of OHT (11 years), the mean SC area in the experimental eyes (550 ± 80 µm²) was markedly smaller than that in the fellow normal eyes 1663 ± 674 µm².

**Conclusions:** Schlemm’s canal can be noninvasively assessed in the monkey eye. These measurements will be useful in physiological studies of aqueous outflow and may have important implications for the development of treatments that target the trabecular outflow pathway.

**Commercial Relationships:** Byron H. Li, Novartis Institutes for Biomedical Research (E); Shenouda Yacoub, Novartis Institutes for Biomedical Research (E); Rad Daly, Novartis Institutes for Biomedical Research (E); Sarah S. Webb, Novartis Institutes for Biomedical Research (E); Brian Thomas, Novartis Institutes for Biomedical Research (E); Quina Sessums, Novartis Institutes for Biomedical Research (E); Glen Jernigan, Novartis Institutes for Biomedical Research (E); Terri Krause, Novartis Institutes for Biomedical Research (E); Ganesh Prasanna, Novartis Institutes for Biomedical Research (E); Dennis S. Rice, Novartis Institutes for Biomedical Research (E)

**Program Number:** 2896 Poster Board Number: B0294
**Presentation Time:** 8:30 AM–10:15 AM
**Comparative IOP lowering in a 7-day repeated dose study of latanoprost, travoprost, bimatoprost, tafluprost, and ONO-9054 in normotensive Monkeys**


**Purpose:** ONO-9054 (Ono Pharmaceuticals, Osaka Japan) is a novel dual FP/EP3 agonist prodrug under development for the treatment of ocular hypertension and glaucoma. The purpose of this study was to investigate the effect of ONO-9054 on intraocular pressure (IOP) in normotensive monkeys during and after 7-day repeated ocular administration and compare the extent of IOP lowering to that produced by latanoprost, travoprost, bimatoprost, and tafluprost.

**Methods:** IOP lowering effects of ONO-9054 (30 µg/mL), latanoprost (50 µg/mL), travoprost (40 µg/mL), bimatoprost (300 µg/mL), and tafluprost (15 µg/mL) were examined in male cynomolgus monkeys. Drops were administered topically in a 30 µL volume once into each eye for 7 days. IOP was measured with an application pneumotonometer just before and at 4, 8, 12, and 24 hours after ocular administration on days 1, 4 and 7 as well as at 48 and 72 hours after the last administration.

**Results:** ONO-9054, latanoprost, travoprost, bimatoprost, and tafluprost caused IOP reductions at 4, 8, 12, and 24 hr after application on days 1, 4, and 7. The maximal IOP reductions achieved with ONO-9054 (5.8 ± 0.7, 6.6 ± 0.7, and 7.3 ± 0.8 mmHg on days 1, 4, and 7, respectively) were greater than those achieved with latanoprost (3.4 ± 0.6, 4.6 ± 0.5, and 4.9 ± 0.4 mmHg), travoprost (4.0 ± 0.5, 4.9 ± 0.5, and 5.1 ± 0.6 mmHg), bimatoprost (1.9 ± 0.4, 3.1 ± 0.4, and 4.0 ± 0.5 mmHg), or tafluprost (3.5 ± 0.8, 4.3 ± 0.7, and 4.6 ± 0.7 mmHg) on the same days. Furthermore, ONO-9054 showed longer-lasting IOP-reducing effects following the last dose on day 7.

**Conclusions:** The IOP-lowering effects of the FP/EP3 agonist ONO-9054 were more potent and longer-lasting than those produced by any of the marketed FP receptor agonists. The possibility that the dual FP/EP3 receptor agonist ONO-9054 may produce a similar advantage in IOP lowering for patients with ocular hypertension or glaucoma is currently under investigation in a phase 2 clinical trial.

**Commercial Relationships:** Shinsaku Yamane, Ono Pharmaceutical Co., Ltd. (E); Tomohiro Karakawa, Ono Pharmaceutical Co., Ltd. (E); Kazumi Moriyuki, Ono Pharmaceutical Co., Ltd. (E); Masafumi Sugitani, Ono Pharmaceutical Co., Ltd. (E); Yasushi Hirota, Ono Pharmaceutical Co., Ltd. (E)

**Program Number:** 2897 Poster Board Number: B0295
**Presentation Time:** 8:30 AM–10:15 AM
**Melatonin, IIK7 and 5-MCA-NAT potentiate adrenergic receptor-mediated ocular hypotensive effects in rabbits:**

Melatonin, IIK7 and 5-MCA-NAT potentiate adrenergic receptor-mediated ocular hypotensive effects in rabbits: significance for combination therapy in glaucoma

Alejandro Martinez-Aguila, Almudena Crooke, Fernando Huete-Toral, Alba Martin-Gil, Begoña Fonseca, Jesús Pinto. Biochemistry and Molecular Biology IV, Complutense University, Madrid, Spain.

**Purpose:** To investigate the possible potentiating effect of melatoninergic drugs upon the ocular hypotensive action of brimonidine, a selective α2-adrenergic receptor agonist (Alphagan), and timolol, a nonselective β-adrenergic receptor antagonist (Timoptic).

**Methods:** Male New Zealand white rabbits, weighing 3–4 kg, were used for IOP studies. IIK7, melatonin and 5-MCA-NAT were formulated in isotonic saline containing 1% dimethylsulfoxide (DMSO) and were tested at a final concentration of 100 µM. Alphagan 0.2% (brimonidine) and Timoptic 0.5% (timolol) were instilled at a fixed volume of 40 µl. Studies of mRNA and protein expression were conducted using immortalized rabbit nonpigmented ciliary epithelial (NPE) cells. Total RNA was isolated using the RNeasy MiniKit (Qiagen), according to the manufacturer’s protocol. Immunofluorescent staining was performed to evaluate α2A- and β2-adrenergic receptor expression in rabbit NPE cells treated with melatonin and 5-MCA-NAT.

**Results:** Intraocular pressure (IOP) experiments showed that the pretreatment with IIK7, melatonin or 5-MCA-NAT increased the hypotensive effect of timolol in 10.84% ± 4.11% (P< 0.05), 14.02% ± 5.8% and 16.75% ± 5.48% (P< 0.01), respectively, in rabbits for 24 hours. Concerning brimonidine hypotensive action, an additional IOP reduction of 32.11% ± 4.68%, 29.26% ± 5.21% and 39.07% ± 5.81% (P< 0.001) were observed in rabbits pretreated with IIK7, melatonin or 5-MCA-NAT, respectively, when compared with animals treated with brimonidine alone for 24 hours. Additionally, a sustained potentiating effect of a single dose of IIK7 and 5-MCA-NAT were seen in rabbits treated with brimonidine once daily for up to 4 days (extra IOP decrease of 16.61% ± 5.12% and 15.57% ± 5.15% (P< 0.05), compared with brimonidine alone).

**Conclusions:** These data confirm the indirect action of melatoninergic compounds on adrenergic receptors and their
remarkable effect upon the ocular hypotensive action mainly of α2-adrenergic receptor agonists but also of β-adrenergic antagonists.

**Commercial Relationships:** Alejandro Martínez-Águila, None; Almudena Crooke, None; Fernando Huete-Toral, None; Alba Martin-Gil, None; Begoña Fonseca, None; Jesus Pintor, None

**Support:** Spanish Ministry of Economy and Competition (Grant SAF2010-16024); Ministry of Health Social Services and Equality RETICS (Grant RD07/0034/003); and Complutense University of Madrid (Grant GR35/10-A-920777). This work also was supported by a Spanish Ministry of Economy and Competition studentship (to F.H.-T.); and Complutense University of Madrid studentships (to A.M.-À. and A.M.-G.).

**Program Number:** 2898 **Poster Board Number:** B0296

**Presentation Time:** 8:30 AM–10:15 AM

**Oxygen Levels and Distribution in Rhesus Monkeys: Association with Intraocular Pressure and Aqueous Outflow Facility**


**Department of Ophthalmology and Visual Sciences, Washington University, St. Louis, MO; **Department of Cell Biology and Physiology, Washington University, St. Louis, MO; **Department of Ophthalmology and Visual Sciences, University of Wisconsin, Madison, WI.

**Purpose:** Previous studies suggested that oxidative stress plays a role in the development of glaucoma and that the lens may protect the outflow system from oxidative damage after vitrectomy. (Chang S, AJO 2006;141:1033) In human subjects, vitrectomy and cataract surgery significantly increase the exposure of the tissues of the outflow pathway to oxygen, potentially increasing the likelihood of oxidative damage in these tissues. (Siegfried C et al, IOVS 2010;51:5731) To explore the impact of vitrectomy and cataract extraction on the function of the aqueous humor outflow pathway, we measured oxygen (pO2), intraocular pressure (IOP) and determined aqueous outflow facility in the eyes of rhesus monkeys undergoing pars plana vitrectomy (PPV) followed by cataract extraction (CE).

**Methods:** Five rhesus monkeys (ages 19-21 years) underwent sequential PPV, CE with lens implantation in one eye and sham procedures in the fellow eye, using standard surgical techniques. Intraocular pO2 measurements were performed with the Oxylab pO2® optical oxygen sensor (Oxford Optronix) in the anterior chamber (AC), posterior chamber (PC) and vitreous cavity, as previously described. IOP and pneumotonomography data were obtained to calculate outflow facility at baseline, post-PPV, and post-CE. Aqueous and vitreous humor specimens were obtained. Comparisons between surgery and sham eyes were performed by the 2-tailed paired t-test.

**Results:** pO2 increased in the AC angle following vitrectomy (p=0.01) and CE (p=0.001) as well as at the lens surface (p=0.001) and the PC following CE (p=0.001). IOP increased following PPV and CE, from baseline mean IOP 19.0 mmHg to 22.3 mmHg, (p<0.05). Although outflow facility decreased in both the sham and surgery eyes, the decline was greater in the surgery eyes.

**Conclusions:** Our preliminary data in this study of the effect of increased oxygen on aqueous outflow facility and IOP suggest a correlation with decreased function of the conventional outflow pathways. The impact of increased exposure to oxygen and potential oxidative damage may be further understood through our pending studies on the antioxidant status of the aqueous and vitreous humor samples as well as histopathological examination of these macaque eyes.
**Purpose:** AR-13324 is a new dual-action compound that is in clinical development for the treatment of glaucoma and ocular hypertension. It is both a Rho kinase (ROCK) inhibitor and norepinephrine transporter inhibitor that has been shown in a primate model to lower IOP through a dual mechanism of action, increasing trabecular outflow facility and decreasing aqueous production. The present study tested the hypothesis that AR-13324 also lowers IOP by reducing episceral venous pressure.

**Methods:** In anesthetized Dutch Belted rabbits (n=6), we measured ear artery pressure (BP) and IOP by direct cannulation; carotid blood flow (BFcar) by transit time ultrasound, heart rate (HR) by a digital cardiotachometer, and EVP with a servo-null micropressure system. Animals were dosed with AR-13324 (0.04%, topical) once-daily for three days. On Day 3, measurements were obtained prior to dosing (Baseline) and for 3 hours following dosing of AR-13324. The data (mean +/- standard error) were analyzed by repeated measures ANOVA with Bonferroni post hoc testing.

**Results:** Baseline values were: BP; 105 ± 3 mmHg, IOP; 34 ± 4 mmHg, EVP; 17 ± 2 mmHg, BFcar; 35 ± 1.1 ml/min, and HR; 337 ± 9 bpm. Three hours post dosing, IOP was reduced by 39 ± 7% (to 19 ± 2 mmHg, p<0.001) and EVP by 35 ± 4% (to 11 ± 1 mmHg, p<0.05). BP, BFcar and HR remained stable throughout the experiment.

**Conclusions:** AR-13324 lowered IOP and EVP significantly in Dutch Belted rabbits. At baseline, half of the measured IOP (34 mmHg) was attributable to EVP (17 mmHg), assuming the 1:1 correspondence between IOP and EVP in the Goldmann equation. At 3 hours post-dosing, the reduction in EVP caused by AR-13324 treatment accounted for 42% of the measured reduction in IOP. The remaining reduction in IOP achieved by AR-13324 is presumed to occur through increased outflow facility and decreased aqueous production, based upon previous mechanism of action studies in cynomolgus monkeys. Also noteworthy, the baseline values for BP, IOP and EVP in the Dutch Belted rabbit were markedly higher than previously reported for the New Zealand rabbit. We conclude that in the Dutch Belted rabbit model, AR-13324 lowers IOP in part through significant lowering of EVP.

**Commercial Relationships:** Jeffrey W. Kiel, Aerie Pharmaceuticals, Inc (F); Casey Kopczynski, Aerie Pharmaceuticals, Inc. (E)

**Support:** Aerie Pharmaceuticals, Inc. and the van Heuven endowment

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**Support:** Aerie Pharmaceuticals, Inc. and the van Heuven endowment

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**Results:** Fluorophotometric aqueous flow with ketamine/xylazine anesthesia was 0.09±0.01 μl/min (meansSEM, n=24). This was significantly slower than with ketamine alone or 2,2,2-tribromoethanol (p<0.001). Aqueous flow could not be measured when isoflurane was used because of eye tremor. Timolol reduced aqueous flow from 0.20±0.02 μl/min to 0.07±0.01 μl/min (p=0.001) under 2,2,2-tribromoethanol anesthesia and from 0.14±0.01 μl/min to 0.10±0.01 μl/min (p=0.004) under ketamine anesthesia but did not reduce aqueous flow significantly under ketamine/xylazine anesthesia. Dorzolamide reduced aqueous flow from 0.09 ± 0.01 to 0.06 ± 0.01 μl/min (P = 0.04) under ketamine/xylazine anesthesia.

**Conclusions:** The advantages of the fluorophotometric method to measure aqueous flow are that it is noninvasive and repeat measurements can be performed on an individual mouse. The method does require anesthesia to sedate mice as the method is sensitive to movement. The type of anesthesia can greatly affect aqueous flow and efficacy of aqueous flow suppressants. When designing an experiment to measure IOP and aqueous humor dynamics in mice, the method of sedation needs to be chosen carefully.

**Commercial Relationships:** Carol B. Toris, None; Shan Fan, None; Cassandra L. Hays, None; Bruce M. Ishimoto, Ocumetrics (E)

**Support:** NIH Grant EY016902 (BI) and Research to Prevent Blindness

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**Purpose:** While currently clinically approved glaucoma treatments target at altering aqueous humor (AH) dynamics to lower elevated intraocular pressure (IOP), the contributions of imbalanced AH dynamics to the pathogenesis of ocular hypertension (OHT) and glaucoma are not fully elucidated. This study used a small-molecular-weight MRI contrast agent, gadolinium (Gd) to mimic AH and visualize AH dynamics in rats upon microbead (MB)-induced OHT or ocular hypotensive drug treatments.

**Methods:** 8 Sprague-Dawley rats were injected with a mixture of 10 and 15μm MB into the right (R) anterior chamber (AC) to block aqueous outflow (AO) and induce sustained OHT for 2 weeks. 18 additional normotensive rats were topically applied to R eye with 0.005% latanoprost (Lat; n=6), 0.5% timolol maleate (Tim; n=6) and 0.2% brimonidine tartrate (BT; n=6) 1hr before MRI to lower IOP by facilitating AO, suppressing aqueous inflow (AI) and both IIR of Gd signal, peak signal intensity (PI) and time to peak (TP) in AC and vitreous body (VB) were computed.
**Results:** Right before MRI, the mean % IOP changes of treated R eye relative to untreated left (L) eye were 100%, -29%, -27%, -36% and -4% for MB, Lat, Tim, BT and Sal groups (p<0.05 except Sal). IOP increase by AO reduction in MB gave higher IIR & PI and shorter TP in R than L AC (p<0.05), whereas IOP decrease by AO increase in Lat or AO reduction in Tim gave opposite Gd-MRI patterns to MB (p<0.05 for Lat, p=0.06-0.2 for Tim). For BT, both L and R AC showed lower IIR, higher PI and longer TP than other groups (p<0.05). Only MB had higher PI in R than L VB (p<0.05). No Gd-MRI difference was found between both eyes in Sal, whereas IIR in L AC was slightly higher in Lat and Tim than MB and Sal (p=0.09-0.16).

**Conclusions:** IIR, PI and TP in Gd-MRI may reflect altered AH dynamics quantitatively upon OHT or anti-glaucoma drug treatments. The drastic Gd-MRI changes in BT and the slight changes in untreated AC of Lat and Tim may also draw attention to the systemic effects of topical hypotensive drugs on AH dynamics.

**Commercial Relationships:** Leon C. Ho, None; Ian P. Conner, None; Chi-wai Do, None; Seong-Gi Kim, None; Ed X. Wu, None; Gadi Wollstein, None; Joel S. Schuman, Zeiss, Inc. (P); Kevin C. Chan, None

**Support:** NIH Grant P30 EY008098 and UL1 TR000005; BrightFocus Foundation G2013077; Eye and Ear Foundation (Pittsburgh, PA); Research to Prevent Blindness.
the current study, we investigated the role of importins-2, 7, 13 and RAN-GTP in mediating dexamethasone-induced RFP-GRx nuclear trafficking.

**Methods:** Stably-transfected RFP-GRx cell lines were developed. siRNAs against importin-2, importin-7, importin-13, and RAN-GTP were transfected in cells and cells were allowed to reach confluency. Nuclear import of RFP-GRx in NTM5 cells treated with vehicle (ethanol) or dexamethasone (DEX) was studied using confocal microscopy and western blot in isolated nuclear and cytosolic fractions. Leptomycin B (an inhibitor of exportin-1), was tested for its ability to block RFP-GRx-nuclear export following DEX removal. **Results:** NTM5 cells transfected with siRNAs against importin-2 and RAN-GTP significantly inhibited DEX-induced nuclear import of RFP-GRx receptor that accumulated in the cytosol after DEX treatment. In contrast, both siRNAs against importin-7 and 13 failed to block DEX-induced nuclear import of RFP-GRx. Leptomycin while not affecting nuclear import of RFP-GRx, attenuated nuclear export of RFP-GRx following DEX removal. **Conclusions:** While DEX-induced RFP-GRx nuclear import is importin-2 and RAN-GTP-dependent, neither importin-7 nor importin-13 appear involved in such pathway. However, exportin-1 appears to mediate nuclear export of RFP-GRx receptor following DEX removal.

**Commercial Relationships:** Adnan Dibas, None; Abbot F. Clark, None; Thomas Yorio, None

**Support:** NIH grant EY016242

**Program Number:** 2905 Poster Board Number: B0303

**Presentation Time:** 8:30 AM–10:15 AM

**Cell culture of trabecular meshwork cells under continuous oxidative stress by photocatalytic generation of H2O2.**

**Shaun P. Garland**, Joshua Morgan, Christopher J. Murphy, Paul Russell

1Biomedical Engineering, University of California, Davis, Davis, CA; 2Department of Surgical & Radiological Sciences, School of Veterinary Medicine, University of California, Davis, Davis, CA; 3Department of Ophthalmology & Vision Sciences, School of Medicine, University of California, Davis, Davis, CA

**Purpose:** Oxidative stress, often particularly mediated by the reactive oxygen species (ROS) H2O2, has been implicated as a component of a great number of disease states including glaucoma. However, the common method by which oxidative stress is studied in vitro is by bolus dosing, which is not biomimetic in concentration or duration. We recently reported a method for tunable, continuous photocatalytic generation of H2O2 in cell culture that much more closely resembles the oxidative stress profile of chronic diseases such as glaucoma. In this work, we have improved the implementation of the ROS generator and investigated the stability of cultures of primary human trabecular meshwork (HTM) cells under continuous ROS levels over 3 d. We wish to determine a ROS generation rate that would not kill HTM cells.

**Methods:** Photoactive hydrogels were synthesized by mixing a 1:1 volume of poly(ethylene glycol) diacrylate (700 g/mol) with 0.25 mg/mL 2,6-anthraquinone sodium sulfate. After crosslinking with UV light, hydrogels were punched to 6 mm discs and sterilized with 70% ethanol. HTM cells were plated 50,000/well in a 24-well plate in Hank’s buffered salt solution. Transwell inserts with photoactive hydrogels were placed in each well. An LED emitting 405 nm light was used to illuminate each well of the experimental groups continuously for 3 d. Cells were then fixed and stained with DAPI and nuclei were counted to determine cell numbers. H2O2 was measured by the xylene orange method.

**Results:** After 3 d, cells densities were 127 ± 10 and 128 ± 10 cells/mm² for control and ROS-exposed groups, respectively. The steady-state concentration of H2O2 was 400 nM without cells, which corresponds to a generation rate of 150 nM/min according to the kinetic model.

**Conclusions:** Following continuous ROS exposure, the HTM cells in the experimental groups had similar cell densities compared to control groups indicating no loss of cells during the culture period. The total dose was 650 µM H2O2 over the duration of the study which would otherwise prove fatal as a bolus. The culture system described here can be used in future studies to examine the cellular response to chronic oxidative loads. This system enables monitoring changes in gene and protein expression levels that provide insights into alterations in the HTM during the progression of glaucoma.

**Commercial Relationships:** Shaun P. Garland, None; Joshua Morgan, None; Christopher J. Murphy, EyeKor LLC (I), Imbed LLC (I), Ocular Services On Demand (C), Ocular Services On Demand (I), Platypus Technologies LLC (I); Paul Russell, None

**Support:** NIH Grant R01EY019475

**Program Number:** 2906 Poster Board Number: B0304

**Presentation Time:** 8:30 AM–10:15 AM

**Myocilin Mutations Alter GPCR Endocytosis**

Trent J. Bowen1, Nicole R. Congrove1, W Daniel Stamer2, Brian S. McKay1

1Ophthalmology and Vision Science, University of Arizona College of Medicine, Tucson, AZ; 2Ophthalmology, Duke University, Durham, NC

**Purpose:** Different mutations in myocilin cause glaucoma with distinct disease severity and age of onset. We previously showed that myocilin participates in G-protein coupled receptor (GPCR) endocytosis in a ligand-dependent manner. In this study, we tested the hypothesis that mutations in myocilin alter its function in endocytosis of GPR143 after ligand stimulation.

**Methods:** COS cells were transfected to co-express an isoform of myocilin (WT, G364V or P370L) and GPR143 (as a GFP fusion protein). GPR143 receptors were stimulated with their endogenous ligand (L-DOPA, 5 µM) for 0, 2, 5, 20, 40, and 60 minutes. The cells were washed with cold PBS and fixed using 4% paraformaldehyde. Fixed cells were permeabilized using TBST and stained using affinity-purified antibodies directed against human myocilin. Myocilin specific staining was visualized using a secondary antibody conjugated to TRITC. Confocal microscopy with a Zeiss LSM 510Meta-NLO multiphoton microscope was performed at 40x magnification at 488 nm and 543 nm excitation wavelengths. GFP and myocilin image stacks were produced separately and overlaid using ImageJ.

**Results:** At time 0 (before ligand stimulation), WT myocilin displayed a diffuse cytoplasmic distribution, whereas GPR143 was both on the plasma membrane and in the Golgi apparatus. As early as 2 minutes after stimulation, the distribution of both myocilin and GPR143 changed, and both proteins localized to early endosomes. The proportion of both proteins localized to the early endosome continued to increase as the time course proceeded, and at 20 minutes both proteins were localized to a large late endosome. By 40 minutes, myocilin and GPR143 no longer co-localized, returning completely to time 0 distribution by 60 minutes. The P370L myocilin mutant did not co-localize with GPR143 after treatment until 40 minutes when both appeared in late endosomes. P370L returned completely to conditions at 0 time 0 conditions at 60 minutes. G364V behavior following treatment was indistinguishable from WT.

**Conclusions:** Our results indicate that the P370L mutation alters myocilin’s function in receptor-mediated endocytosis. The G364V mutation does not alter its function in endocytosis, yet both P370L and G364V mutations cause glaucoma. These observations suggest that mutations in myocilin differentially affect receptor-mediated...
endocytosis which may account for the distinct disease severity and age of onset seen with glaucoma involving mutations in myocilin.

Commercial Relationships: Trent J. Bowen, None; Nicole R. Congrove, None; W Daniel Stamer, None; Brian S. McKay, None

Support: RPB: Center Grant

Program Number: 2907 Poster Board Number: B0305
Presentation Time: 8:30 AM–10:15 AM

Strain Survey of Aqueous Humor Dynamics in the Mouse

J Cameron Millar1, 2, Iok-Hou Pang3, Abbot F. Clark1, 2, Cell Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX; 2North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, TX; 3Department of Pharmaceutical Sciences, University of North Texas Health Science Center, Fort Worth, TX.

Purpose: Recently mice have been extensively used as an experimental model for glaucoma. The mouse eye bears many similarities to the human in terms of anatomy, physiology, and pharmacology. However, its small size presents a challenge for the study of aqueous humor dynamics (AHD). Mouse AHD parameters have been studied by several laboratories in various strains, but there is no clear consensus. We hypothesize that strain differences may play a role. This study was designed to evaluate potential differences in AHD among several commonly used mouse strains with or without ocular hypertension induced by intraocular injection of a viral vector encoding mutant myocilin.

Methods: Female BALB/cJ, A/J, and C57- BL/6J mice, aged 12-25 weeks and weighing between 21 and 28g were utilized. Animals were fed standard chow and kept in 12h light/12h dark conditions (lights on @0600hrs). Intracocular pressure (IOP) was elevated in the left eye by intravitreal injection of Ad5.MYOC.Y437H. The right (naïve) eye was not injected. IOP was measured using a Tonolab tonometer (ICare®). AHD parameters (outflow facility (C), episcleral venous pressure (Pe), aqueous humor inflow rate (Fin), and uveoscleral outflow rate (Fu)) were established by constant flow infusion in anesthetized animals following our previously published methodology (Millar et al., IOVS 52:685-694, 2011).

Results: Intravitreal injection of Ad5.MYOC.Y437H significantly increased IOP in BALB/cJ mice (P<0.001), A/J mice (P=0.001) and C57- BL/6J mice (P<0.001). Mean C in A/J mouse eyes was greater than that of BALB/cJ mouse eyes (1.24× (naïve); 1.21× (injected)), and mean C in C57- BL/6J mouse eyes was greater than that of BALB/cJ mouse eyes (1.52× (naïve); 1.26× (injected)). In each strain, C in eyes injected with Ad5.MYOC.Y437H was significantly reduced, by a factor of 1.32× (BALB/cJ), 1.35× (A/J) and 1.58× (C57- BL/6J) compared with uninjected contralateral eyes (P=0.00716, ANOVA). Fu and Pe were not found to be significantly different between the BALB/cJ and the A/J strains, nor between the naïve or Ad5.MYOC.Y437H-injected eyes within these strains.

Conclusions: There are strain differences in C but not in other AHD parameters. Intravitreal injection of a vector encoding mutant myocilin increased IOP mainly due to a reduction in C.

Commercial Relationships: J Cameron Millar, None; Iok-Hou Pang, None; Abbot F. Clark, None
Support: IADR Grant R16071

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splice variants that have opposing effects on VEGF receptor stimulation. This project aims to determine the effect of VEGF_{165a} and VEGF_{165b} on conventional outflow facility (C) in mice.

**Methods:** Enucleated eyes from 24 C57BL/6J mice (10-12 weeks old, male) were perfused ex vivo using a pumpless perfusion system optimized for mouse eyes. An inline flow sensor was used to measure the flow rate (Q) into the eye as IOP was varied in steps of 2.5 mmHg between 5 and 20 mmHg using an adjustable height reservoir. C was calculated as the slope of the linear regression of Q versus IOP.

**Results:** VEGF_{165a} dose-dependently increased C. In controls, the baseline C was 13.59±7.6 nL/min/mmHg (N=17 eyes). VEGF_{165a} increased C by 37±21% at 0.1 μg/mL (p=0.04, N=6) and by 58±23% at 0.5 μg/mL (p=0.02, N=4). In contrast, VEGF_{165b} decreased C by 62±38% (p<0.0005; N=5) at 0.5 μg/mL.

**Conclusions:** VEGF splice variants have differential effects on conventional outflow in mice, with VEGF_{165a} increasing C and VEGF_{165b} decreasing C. The balance between VEGF_{165a} and VEGF_{165b} may thereby regulate trabecular outflow and may serve as a target for future glaucoma therapies. Patients on long-term anti-VEGF therapy may therefore experience compromised outflow function resulting from an imbalance in VEGF isoforms levels in the trabecular meshwork.

**Commercial Relationships:** Ester Reina-Torres, None; Joseph M. Sherwood, None; W Daniel Stamer, None; Darryl R. Overby, None

**Support:** Fight for Sight PhD Studentship (Ref 1385)

**Program Number:** 2910 Poster Board Number: B0308

**Presentation Time:** 8:30 AM–10:15 AM

**Role of VEGF in Conventional Outflow Homeostasis**

Katy C. Liu¹, Guorong Li², Darryl R. Overby³, W Daniel Stamer³
¹University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC; ²Department of Ophthalmology, Duke University, Durham, NC; ³Department of Bioengineering, Imperial College London, London, United Kingdom

**Purpose:** Vascular endothelial growth factor (VEGF) is a secreted protein with a variety of functions including angiogenesis, vasodilation, and increased membrane permeability. Intravitreal anti-VEGF injection is the current mainstay treatment for many retinal diseases. Of recent concern, anti-VEGF injections have resulted in an alarming increase in intraocular pressure (IOP) in a subset of patients, the mechanism of which is unknown. Currently, there is little understanding of VEGF and its roles in the outflow tract and glaucoma. In this study, we characterize endogenous and stress-induced VEGF secretion in the outflow tract, and we test the hypothesis that VEGF increases outflow facility in vivo.

**Methods:** Human trabecular meshwork (TM) and Schlemm’s canal (SC) cells were isolated and grown in culture. Endogenous VEGF levels (isoform 165) were quantified by ELISA from cell supernatants. TM cells were plated onto flexible membranes, and cyclic mechanical stretch (16% elongation, 1 cycle/s) was applied for 24 hours (Flexcell); or TM cells were treated with 100 nM dexamethasone (dex) for up to 7 days. To determine the effect of VEGF on SC cell permeability, SC cells were grown to confluence on transwell filters, and transendothelial electrical resistance (TEER) was measured in response to VEGF (25, 100 ng/mL). To study the effect of VEGF on outflow facility, VEGF (100 ng/mL) or PBS was infused into the anterior chambers of living mice. Flow was measured at fixed pressures (15, 25, 35 mmHg), and conventional outflow facility was calculated using Goldmann’s equation.

**Results:** VEGF is secreted by both TM and SC cells in culture. In response to mechanical stretch, TM cells increased VEGF secretion by 44% (p<0.02). Similarly, VEGF levels were higher in response to dex at 3, 5, and 7 days (501±106 ng/mL VEGF vs 329±18 ng/mL control, p<0.05), and as a control, myocilin expression increased in dextreated cells. Interestingly, SC monolayers showed no change in TEER in response to VEGF, while VEGF resulted in a 25% increase (p<0.04) in outflow facility in vivo.

**Conclusions:** Our study demonstrates that cells of the conventional outflow tract secrete endogenous levels of VEGF that increase with stretch. Importantly, exogenous VEGF increases outflow facility in vivo, suggesting that anti-VEGF therapy may affect outflow regulation and IOP by altering endogenous VEGF tone.

**Commercial Relationships:** Katy C. Liu, None; Guorong Li, None; Darryl R. Overby, None; W Daniel Stamer, None

**Support:** NIH F30DK089695, NIH EY022359

**Program Number:** 2911 Poster Board Number: B0309

**Presentation Time:** 8:30 AM–10:15 AM

**Repeatability of Episcleral Venous Pressure Measurement**


**Purpose:** Episcleral venous pressure (EVP) is an important determinant of intraocular pressure (IOP) and can be measured by estimating the pressure required to compress an episcleral vein to a predetermined endpoint. This study evaluates the repeatability of EVP measurement in proximal and distal segments of an episcleral vein, two adjacent veins in the same field of view, and two separate measurements in different sessions.

**Methods:** Episcleral venous pressure (EVP) was measured in 17 eyes of 17 patients by using a computer-controlled automated episcleral venomanometer that recorded a video sequence of the vessel as it was compressed. The measured applied pressure was synchronized with the video stream and image analysis software was used to determine the pressure that collapsed a selected segment of an episcleral vein to a predetermined endpoint. In high-quality video recordings, we compared EVP in a proximal segment to EVP in a distal segment of the same vein and EVP between two adjacent veins in the same field of view. We also compared EVP from two measurement sessions in the same eye that were 2-4 hours apart. Significances of differences were determined by using paired t-tests and relationships between measurements were illustrated by Pearson correlation. Limits of agreement between measurements were calculated and defined as the mean difference ± 2 standard deviation (SD) of the difference.

**Results:** Mean EVP in proximal segments (7.9±3 mmHg; ± SD) was not different from mean EVP in distal segments (8.0±2.8 mmHg; p=0.72). EVP in the two segments were correlated (r=0.98, p<0.001, n=10) and limits of agreement were -1.4 to 1.2 mmHg. Mean EVP in the two adjacent vessels of the same field (6.9±2.4 mmHg and 6.6±2.2 mmHg) were not different from each other (p=0.16). EVP in the two vessels were correlated (r=0.98, p<0.001, n=10) and limits of agreement were -0.7 to 1.2 mmHg. Mean EVP was 6.0±2.3 mmHg and 6.1±2.3 mmHg in the first and second measurement sessions respectively (p=0.79) and were correlated (r=0.96, p<0.001, n=17), and limits of agreement between these measurements were -1.4 to 1.3 mmHg.

**Conclusions:** EVP measurements are consistent between different segments along the same vein and between different veins in the same field. Repeated measurements of EVP within the same eye separated by 2-4 hours are also consistent to within less than 1.5 mmHg.
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Automated Assessment of Episcleral Venous Pressure during Venomanometry
Purpose: Episcleral venous pressure (EVP) is determined from the applied pressure that begins to collapse an episcleral vein. However, identifying this endpoint from a video and pressure recording during venomanometry is time consuming. In this study we developed an automated method to determine the pressure that initiates vascular collapse.

Methods: EVP was measured by using a custom computer-controlled episcleral venomanometer in 57 eyes from 29 normal participants. An episcleral vein was recorded by video microscopy through a transparent silicone bulb over the vein while the bulb was inflated linearly to 22 mmHg. After low-pass and homogenization filtering of the green (red-free) image channel in each frame, the average brightness profile across a 0.5 – 1 mm segment of vein was determined by using a custom program, and the peak brightness difference from the center to the edge of the vessel was recorded. A sigmoid function was fitted to the peak brightness vs applied pressure and EVP was assumed to be the pressure where this curve decreased to 0.935 of the initial peak brightness (automated method). EVP was also determined manually by identifying the initial stable peak brightness and the pressure at the beginning of the decrease of peak brightness, determined by back-projection. The automated EVP was compared with manual EVP by using generalized estimating equation models to account for possible correlation between fellow eyes of the same subjects. The relationship between methods was illustrated by Pearson correlation, and limits of agreement were expressed as the mean difference ± 2 standard deviations of the difference.

Results: Mean EVP was 6.6 ± 2.9 mmHg (± SD) and 6.7 ± 2.7 mmHg by using the automated and manual methods respectively (p=0.46). The two methods were correlated (r=0.96, p<0.001); the mean difference was -0.1 ± 0.8 mmHg and the limits of agreement were -1.7 to 1.5 mmHg. The pattern of vascular collapse was not consistent among trials. A biphasic collapse with an initial slow phase followed by a fast phase was noted in 22 of 57 trials. Vessel brightness was variable during the initial stable phase in 36 trials and during the transition in 13 trials.

Conclusions: Use of the fitted sigmoid curve provides a fast and accurate method to determine EVP from the vessel brightness in episcleral venomanometry. However, variability of vascular collapse must be considered in all methods of analysis.

Purpose: The primary aim of this study was to measure the pressure difference between the anterior chamber and the vitreous cavity, which had been predicted with studies with mathematical models, in real eyes.

Methods: This study was conducted using vitrectomized porcine eyes. Infusion pressures of 10 to 80 mmHg were generated with vented gas forced infusion system. Measurement of pressure were obtained with digital manometry connected with 25-gauge catheters from the anterior chamber and vitreous cavity, simultaneously. After increasing the pressure of the anterior chamber to each target pressure, pressure change in the vitreous cavity were recorded, and vice versa.

Results: The obtained intravitreal pressure was similar to the increased intracameral pressure in all cases. The obtained intracameral pressure was similar to the increased intravitreal pressure, under 50 mmHg. When increasing the intravitreal pressure to 60, 70 and 80mmHg, the obtained intracameral pressure were 57.6 ± 1.0, 64.0 ± 0.8, and 69.6 ± 2.4 mmHg, respectively. The obtained intravitreal pressures were 1.5, 5.9 and 9.1 mmHg higher than that of the obtained intracameral pressure with target pressures of 60, 70, and 80mmHg, respectively (P = 0.027, 0.001, 0.001). Pupillary block was observed in case of increasing the intravitreal pressure over 50 mmHg.

Conclusions: The intracameral pressure could be significantly lower than intravitreal pressure in some eyes with pupillary block. When we use the non-invasive tonometer, the possibility of underestimation of operative pressure at the optic nerve head should be considered in eyes with pupillary block.

Figure 1. Obtained pressure from vitreous cavity (VC) or anterior chamber (AC) with various pressure setting of the anterior chamber or vitreous cavity.
Figure 2. Pressure transmission from the vitreous cavity to the anterior chamber in normal eye (left) and eye with pupillary block (right). Aqueous humor can freely flow to the anterior chamber and the vitreous cavity in normal eye (arrows). In the eyes with pupillary block, lens and iris act as a flexible physical barrier for aqueous flow. Higher pressure in vitreous cavity is partially transmitted to anterior chamber not directly through the fluid molecule itself, but only indirectly through the bulging of the iris (dotted arrows).

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Dynamic alterations in conventional outflow function in Bmp2-induced ocular hypertensive mice
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**Purpose:** The goal of this study was to evaluate changes in outflow facility and morphology of the outflow pathway by Spectral Domain Optical Coherence Tomography (SD-OCT) in mice after induction of glaucoma by delivery and overexpression of Bmp2 (bone morphogenetic protein 2) gene to conventional outflow cells.

**Methods:** An adenovirus expressing Bmp2 gene driven by CMV promoter was delivered intracameraly to mice. Intraocular pressure (IOP) was measured pre- and post-infusion twice a week for 36d by rebound tonometry. Outflow facility and IOP was measured by direct cannulation of anterior chamber at 7d, 14d and 36d after viral infusion in living mice. Morphology of outflow tract was followed by SD-OCT and standard histology. Retinal ganglion cell (RGC) health was evaluated by Brn3a labeling of retina flat mounts and axon counts of optic nerves.

**Results:** Overexpression of Bmp2 significantly increased IOP in a biphasic manner; reaching a maximum of ~40 mmHg at day 10, recovering to ~25 mmHg on day 20 and elevating to ~30 mmHg until day 36. IOP in the contralateral control eyes measured ~13 mmHg at all time points. Outflow facility decreased significantly at 7d (0.0049 ± 0.0018 μl/min/mmHg vs 0.0024 ± 0.0012, p=0.034, n=4), 14d (0.0041 ± 0.00096 vs 0.0014 ± 0.00048, p=0.0495, n=3) and 36d (0.0032 ± 0.0019 vs 0.0018 ± 0.0017, p=0.038, n=4). OCT analyses of Bmp-2 expressing eyes showed increased thickness of the cornea (95 ± 4.24 μm vs 119.5 ± 26.16, n=2) and trabecular meshwork (TM) (16 ± 2.83 μm vs 22 ± 2, n=2). Histological data confirmed the OCT findings. OCT also showed that Schlemm’s canal (SC) was less responsive to IOP pressure steps (IOP from 10 to 15 mmHg, SC area reduced 31% in control and 10.4% in Bmp2 eyes, n=2) at 10d. There were significantly fewer RGC bodies in peripheral retinal and fewer axons in optic nerve Bmp2 expressing eyes at 36d.

**Conclusions:** The increase in IOP induced by Bmp2 gene delivery to the mouse anterior chamber may result from the accumulation of ECM in outflow pathway, leading to increased stiffness of TM and compromised homeostatic responsiveness to IOP. Further investigations are needed to determine the mechanism of Bmp2-induced increase in outflow resistance, and value of Bmp2 overexpression as a mouse glaucoma model.

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Six months study on signs and symptoms of polyquad preserved travoprost/timolol fixed combination on previously treated glaucoma patients
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Purpose: To assess usefulness and tolerability of switching glaucoma patients to the fixed combination Travoprost 0.004%/Timolol 0.5% with polyquad (PQ-TTFC); to record effects on tear film break-up time (TF-BUT) and on quality of life (OSDI).

Methods: Multicenter, observational cohort, 6 month study. 50 patients on concomitant Timolol 0.5% (twice a day) and Dorzolamide (twice a day) or Timolol and Latanopost (once a day) (BAK-preserved) were switched to PQ-TTFC (evening dosage). IOP, TF BUT and AEs were recorded and all patients completed the OSDI questionnaire at baseline and after 6 months. All analysis refers to right eye; left eye’s data are similar.

Results: Median age was 70 [63-73] years, women were 54.9% of the sample. IOP significantly decreased (from 18 [16-21] to 15 [12-17] mmHg) after substitution (p<0.001). At baseline 31.4% of patients presented an IOP <18 mmHg, the percentage increased to 80% of subjects at 6 months (p<0.001). TF-BUT improved of 2.7±1.9 sec (from 7 [5-8] to 10 [9-11] sec, p<0.001). Only one patient discontinued the new therapy due to periocular skin pigmentation. Quality of life improved from 28 (moderate) to 19 (mild) (p<0.001).

Conclusions: PQ-TTFC appeared useful in this selected population: patients who underwent a regimen modification to PQ-TTFC obtained further reduction in IOP; improvement in ocular surface status with no exposition to BAK toxicity; and reported a better quality of life perception. The low discontinuation's rate at 6 months indicates a good tolerability profile.