449 Pharmacological Intervention and Cellular Mechanisms

Wednesday, May 10, 2017 11:00 AM–12:45 PM
Exhibit/Poster Hall  Poster Session

Program #/Board # Range: 4587–4615/B0288–B0316
Organizing Section: Glaucoma

Program Number: 4587 Poster Board Number: B0288
Presentation Time: 11:00 AM–12:45 PM

Preoperative brimonidine tartrate 0.2% effect on intraocular pressure of patients undergoing robot-assisted radical laparoscopic prostatectomy in steep Trendelenburg position

Rana Greene, Graham E. Trope, Matteo Parotto, Antonio Finelli, Numan Hallaji, Yaping Jin, Yvonne M. Buys. Ophthalmology, University of Toronto, Toronto, ON, Canada.

Purpose: Postoperative visual field (VF) defects and vision loss secondary to intraocular pressure (IOP) spikes have been reported with robot-assisted radical laparoscopic prostatectomy (RALP) in steep Trendelenburg position (sTBURG). This study evaluated the effect of preoperative brimonidine on IOP during RALP in sTBURG.

Methods: In this prospective randomized controlled masked interventional trial (# NCT02818816), patients scheduled for RALP in sTBURG at University Health Network were screened. Exclusion criteria included diagnosis of glaucoma or ocular hypertension. With informed consent, subjects were randomized to right or left eye. Study eyes were randomized to placebo (artificial tears) or drug (brimonidine tartrate 0.2%) preoperatively. Comprehensive eye exam was done preoperatively and 1-month postoperatively including visual acuity (VA), tonometry, disc photography, VF and retinal nerve fiber layer (RNFL) assessments. A standardized anesthesia protocol was followed intraoperatively. Tono-Pen IOP measurements were recorded bilaterally as follows: pre-anesthesia, anaesthetized supine, and awake supine. The mean of 3 IOP readings each within 2 mmHg was recorded with 5% confidence interval. Primary outcome was IOP. Secondary outcomes were changes in VA, VF and RNFL. Planned sample size is 26 subjects. Study was approved by University Health Network Research Ethics Board.

Results: To date, 5 of 7 recruited patients completed the study (2 brimonidine, 3 placebo). Significant IOP elevation was noted in control, placebo & drug treated eyes at 1 hour of sTBURG. IOP remained elevated during sTBURG. Mean IOP during sTBURG was 31.4±3.6, 30.8±2.4 and 35.9±6.7 mmHg in control, placebo and drug treated eyes respectively, p=0.11 for drug versus placebo. Highest IOP recorded was 44 mmHg at 1 hour of sTBURG, an IOP spike of 30 mmHg from baseline in a drug treated eye. IOP returned to baseline in 8 of 10 eyes 30 minutes after supine positioning. No significant changes were noted in VA, VF or RNFL.

Conclusions: Significant, sustained IOP increase occurs during sTBURG. Early data suggests preoperative brimonidine may not prevent IOP spikes. Enrolment is ongoing.

Commercial Relationships: Rana Greene, None;
Graham E. Trope, None; Matteo Parotto, None; Antonio Finelli, None; Numan Hallaji, None; Yaping Jin, None; Yvonne M. Buys, None

Clinical Trial: NCT02818816

Program Number: 4588 Poster Board Number: B0289
Presentation Time: 11:00 AM–12:45 PM

Glaucoma Eyedrop Instillation Profile in Patients Evaluated in Public and Private Practice Sites

Jose A. Paczka1, Monica Atilano2, Montserrat Romo Sainz3, Luz A. Giorgi Sandoval4, Yesenia Y. Dorantes Diez1. 1Oftalmología, University of Guadalajara, Zapopan, Mexico; 2Research and Development, Unidad de Diagnostico Temprano del Glaucoma, Guadalajara, Mexico; 3Oftalmología, Hospital Dr. Valentín Gómez Farias ISSSTE, Guadalajara, Mexico; 4Research and Development, Asistencia e Investigación en Glaucoma, Guadalajara, Mexico.

Purpose: This study evaluated the performance profile to administer topical hypotensive medications of patients with glaucoma attending either a public hospital or a private specialized glaucoma center.

Methods: This prospective study included consecutive patients with glaucoma visiting either the department of ophthalmology in a large tertiary public hospital or a private referral glaucoma center. Patients completed an 11-item questionnaire regarding aspects of patients’ eye drop administration. Attending ophthalmologists verbally applied such questionnaire to the participants at the time of their regular clinical visit. In addition to providing demographic and general data, relevant information included type of glaucoma, best-corrected visual acuity, visual field indexes, number of glaucoma medications and time since first use of glaucoma eye drop administration ever.

Results: A total of 129 questionnaires were applied to 60 patients at the public hospital and 69 patients at the private glaucoma center. All patients self-administered their drops and had a diagnosis glaucoma during a mean of 4.9 ± 3.1 and 5.6 ± 3.5 years (public site vs. private site, respectively). The proportion of female participants was statistically larger in the private site (P<0.02) as compared to the public one. No statistically difference (P<0.05) was found regarding age (65.9 ± 9.9 vs. 67.3 ± 12.9 years), type of glaucoma, level of education, mean best-corrected visual acuity (LogMAR), mean DM, mean DSM, time using glaucoma drops, and number of glaucoma medications, according to the kind of practice site. On respect of eye drop technique as described by the specific applied questionnaire: a larger proportion of patients attending the private site reported to receive prior information on how to instill eye drops (P<0.0001), and remember to shake the bottle before use (P<0.01); in contrast, a larger proportion of patients attending the public site informed to often have the drops missing the eye (P=0.005).

Conclusions: Differences on specific aspects of self-administration glaucoma eye drops were demonstrated in this study in patients attending either public or private practices. Recognizing the reasons for such differences and the significance of the findings to the process of disease control warrants further investigation at different clinical settings.

Commercial Relationships: Jose A. Paczka, None; Monica Atilano, None; Montserrat Romo Sainz, None; Luz A. Giorgi Sandoval, None; Yesenia Y. Dorantes Diez, None

Program Number: 4588 Poster Board Number: B0290
Presentation Time: 11:00 AM–12:45 PM

Comparative Chromatographic Analysis of Aqueous Mushroom Extracts (Pleurotus tuberregium) with Other Antiglaucoma Drugs

Ghalib A. Akinlabi. Department of Optometry, University of Benin, Benin City, Nigeria.

Purpose: Experimental studies have shown that extracts of Pleurotus tuberregium caused a decrease in intraocular pressure in steroid induced ocular hypertension in feline animal models and a contractile effect on isolated bovine iris. There is therefore an emerging interest in the extract of Pleurotus tuberregium as a potential antiglaucoma

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drug. In this study, a comparative chromatographic analysis and pharmacodynamic study of the activity of aqueous extract of *Pleurotus tuberregium* of various concentrations with known antiglaucoma drugs (0.5% betaxolol, 0.5% timolol maleate, 2% pilocarpine, and 0.005% latanoprost) was carried out.

**Methods:** Graded concentrations (5mg/ml, 10mg/ml, 20mg/ml and 40mg/ml) of the extracts were spotted along with the drugs on prepared glass plates. Silica type 60 gel served as the stationary phase while a solvent system of 50ml chloroform and 50ml absolute ethanol was used as the solvent system. On completion, comparison was made between the extracts and the drugs based on their migration speeds and retardation factors. Further analytic studies were carried out on the filtrates gotten from thin layer chromatography with an Ultraviolet-Visible spectrophotometer.

**Results:** Thin layer chromatographic (TLC) analysis showed that the extracts compared favourably with the drugs under study, particularly with timolol maleate and latanoprost, as they had similar migration distances and retardation factors. A plot of the absorption spectrum of the extract was obtained and compared with the known absorption spectrum of the antiglaucoma drugs under study based on the wavelength at which absorption was maximum. Spectrophotometric studies on the extract revealed that it has an absorption spectrum within the ultra-violet wavelength range with a λ max of 260nm and was compared with the absorption spectra of antiglaucoma drugs under study as determined by previous literatures.

**Conclusions:** The similarities in the RF values of the extract and the known anti-glaucoma drug has been used as a first tier structure activity relationship of the plant extract. There appear therefore to be a promise in the use of the aqueous extract of pleurotus tuberregium in lowering intraocular pressure. Results are presented in tables, and graphs.

**Table 1:** A table showing the migration distances of various concentrations of PT and 0.5% Timolol and 0.05% Latanoprost.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Migration distance</th>
<th>Retardation factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>10mg/ml</td>
<td>6.80cm</td>
<td>0.453</td>
</tr>
<tr>
<td>20mg/ml</td>
<td>7.80cm</td>
<td>0.200</td>
</tr>
<tr>
<td>40mg/ml</td>
<td>10.0cm</td>
<td>0.670</td>
</tr>
<tr>
<td>0.5% Timolol</td>
<td>9.10cm</td>
<td>0.606</td>
</tr>
<tr>
<td>0.05% Latanoprost</td>
<td>10.30cm</td>
<td>0.678</td>
</tr>
</tbody>
</table>

**Commercial Relationships:** Ghalib A. Akinlabi, None

**Program Number:** 4590 Poster Board Number: B0291
**Presentation Time:** 11:00 AM–12:45 PM

**New Highly Effective and Long-Acting Anti-Glaucoma Drug, New Periorbital Delivery Method**

David F F. Woodward1, Jenny W. Wang1, Bioengineering, Imperial College London, London, United Kingdom; 2JeniVision Inc., Irvine, CA.

**Purpose:** Two features define the future of glaucoma therapeutics: (1) greatly improved ocular hypotensive efficacy (2) a delivery method that improves patient convenience and compliance. Periorbital delivery was contemplated since published ocular drug biodisposition studies demonstrate that the overwhelming majority of drugs applied topically to the ocular surface preferentially accumulate in the sclera and eyelids. These studies were intended to determine whether dermal periorbital delivery of an exceptionally efficacious and potent ocular hypotensive agent 3-[(3’–fluoro-4-fluorobiphenyl-3-carbonyl) amino] phenoxyacetic acid isopropyl ester would fulfill the required criteria for a next generation anti-glaucoma drug.

**Methods:** Intraocular pressure was measured in ocular hypertensive and normotensive eyes of conscious cynomolgus monkeys, trained to accept pneumotonometry when under gentle restraint. For periorbital application the compound was formulated in polyethylene glycol and applied radially by using a roller-ball device connected to a cylindrical reservoir.

**Results:** A single 0.006% dose of 3-[(3’–fluoro-4-fluorobiphenyl-3-carbonyl) amino] phenoxyacetic acid isopropyl ester, given as a single eye drop, produced a profound decrease in intraocular pressure in “glaucomatous” monkeys that persisted for one-two weeks. It was not uncommon for a single 0.006% or 0.01% eye drop to reduce intraocular pressure to 6-7 mmHg. Application of a 0.1% dose to the periorbital dermis of ocular normotensive monkeys produced a similarly profound reduction in intraocular pressure, which was well maintained.

**Conclusions:** The compound 3-[(3’–fluoro-4-fluorobiphenyl-3-carbonyl) amino] phenoxyacetic acid isopropyl ester possesses efficacy and duration of action properties sufficient to be considered as representative of the next generation of anti-glaucoma agents. Moreover, application to the periorbital skin using a roller-ball device would be a more convenient method of ophthalmic drug delivery than eye drops and is non-invasive in contrast to other “dropless” technologies.

**Commercial Relationships:** David F F. Woodward, JeniVision Inc. (1); Jenny W. Wang, JeniVision Inc. (1)

**Program Number:** 4591 Poster Board Number: B0292
**Presentation Time:** 11:00 AM–12:45 PM

**Long-term intraocular Pressure (IOP) control by means of a novel biodegradable intracameral (IC) latanoprost free acid (LFA) implant**

Andras M. Komaromy1, Kristin Koehl1, Christine D. Harman1, Gabriel Stewart1, Noah Wolinski2, Taylor N. Norris1, David Valade3, Igor Chekhtman2, John N. Lambert1, Andrew C. Donohue1, Russell Tait2. 1Small Animal Clinical Studies, Michigan State Univ, Coll of Vet Med, East Lansing, MI; 2PolyActiva Pty. Ltd., Melbourne, VIC, Australia.

**Purpose:** Continuous long-term IC drug release represents a promising solution to prevent accelerated progression of glaucomatous optic neuropathy due to low compliance rates for self-administration of IOP-lowering eye drops. PolyActiva cross-linked hydrogel glaucoma implants release LFA continuously for up to 6-months. The purpose of this study was to evaluate long-term implant efficacy in dogs with primary open-angle glaucoma.
**Methods:** LFA implants (220 ng/d) were administered IC via 27G cannula in 10 glaucomatous male and female dogs (ages: 2.8 +/- 1.1 yrs) with IOPs of 24.3 +/- 3.6 SEM mmHg. These implants had 3 different chemistries (PA5004: n=5 eyes; PA5108: n=5; PA5024: n=4) a common diameter of 0.2 mm and maximum segment lengths of 7mm. The implants were evaluated for duration of efficacy and biodegradation. The dogs were followed by routine ophthalmic examination. The primary efficacy outcome measures were diurnal IOP (Icare® TONOVET) and pupil diameter. Xalatan® (0.005% latanoprost) drops (n=4) administered topically q24h and IC blank implants (n=4) served as positive and negative controls for 24 wks, respectively. Treatment effects were compared between groups and to baseline by Student’s t-test.

**Results:** All 3 LFA-implants lowered IOP comparable to Xalatan®; however, the implants reduced the diurnal IOP fluctuations. Degree of pupil constriction closely mirrored the IOP responses. For PA5024, IOPs were significantly lower than baseline at the primary time-points of 4 (11.7 +/- 2.9 SEM mmHg, p=0.003), 8 (12.3 +/- 1.7 SEM mmHg, p=0.003), 16 (13.9 +/- 1.8 SEM mmHg, p=0.007) and 24 wks (16.0 +/- 3.0 SEM mmHg, p=0.088), and IOP drop (≥30%) continued for 34 wks. With PA5004 and PA5108 this IOP-lowering effect lasted for 10 and 19 wks, respectively. Gonioscopy confirmed gradual implant biodegradation (PA5004: 10 wks; PA5108: 23 wks) coinciding with loss of therapeutic effect. Blank implants had no therapeutic effect. All implants were well tolerated with no clinical signs of intraocular inflammatory response.

**Conclusions:** Continuous IC LFA release is an effective and safe means for long-term, stable IOP control. The treatment period can be tailored by varying the implant chemistries. The accelerated biodegradation once drug release has subsided represents an ideal implant characteristic.

**Commercial Relationships:** Andras M. Komaromy; Kristin Koehl; None; Christine D. Harman; None; Gabriel Stewart; None; Noah Wolinski; None; Taylor N. Norris; None; David Valade; PolyActiva Pty. Ltd. (E); PolyActiva Pty. Ltd. (P); PolyActiva Pty. Ltd. (I); Igor Chekhman; PolyActiva Pty. Ltd. (E); PolyActiva Pty. Ltd. (F); John N. Lambert; PolyActiva Pty. Ltd. (E); PolyActiva Pty. Ltd. (I); Andrew C. Donohue; PolyActiva Pty. Ltd. (E); PolyActiva Pty. Ltd. (P); PolyActiva Pty. Ltd. (I); Russell Tait; PolyActiva Pty. Ltd. (E); PolyActiva Pty. Ltd. (S); PolyActiva Pty. Ltd. (P), PolyActiva Pty. Ltd. (I).

**Program Number:** 4593 Poster Board Number: B0294

**Presentation Time:** 11:00 AM–12:45 PM

**Safety of a novel biodegradable intracameral (IC) latanoprost free acid (LFA) implant for long-term intraocular pressure (IOP) control**

**Kristin Koehl¹, Christine Harman¹, Gabriel Stewart¹, Noah Wolinski¹, Taylor N. Norris¹, David Valade¹, Andrew C. Donohue³, Igor Chekhman¹, John N. Lambert¹, Russell Tait¹, Andras M. Komaromy¹.** ¹Small Animal Clinical Sciences, Michigan State University, East Lansing, MI; ³PolyActiva Pty. Ltd., Melbourne, VIC, Australia.

**Purpose:** Purpose: Continuous long-term IC drug release represents a promising solution to prevent accelerated progression of glaucomatous optic neuropathy due to low compliance rates for self-administration of IOP-lowering eye drops. PolyActiva cross-linked hydrogel glaucoma implants release LFA continuously for up to 34 wks. By varying the polymer chemistry, we have shown effective and selective control of IOP for 10, 20 or 34 wks in dogs with primary open-angle glaucoma by changing the period of implant biodegradation. The purpose of this study was to evaluate safety of the implants in these dogs.

**Methods:** Methods: LFA-releasing implants (220 ng/d) were administered IC via 27G cannula in 10 glaucomatous male and female dogs (ages: 2.8 +/- 1.1 yrs). The implants used 3 different biodegradation chemistries (PA5004: n=5 eyes; PA5108: n=5; PA5024: n=4) to set the implant treatment period. The implants had a common 0.2mm diameter and maximum segment lengths of 7mm. Safety was evaluated regularly by ophthalmic examination, pachymetry, visual acuity (obstacle course), and optical coherence tomography (OCT, Spectralis®, Heidelberg Engineering). Electroretinograms were recorded at the end of the study. Xalatan® (0.005% latanoprost) drops (n=4) administered topically q24h and IC blank implants (n=4) served as positive and negative controls, respectively.

**Results:** Results: Implants resided in the inferior iridocorneal angle with no evidence of migration. They were well tolerated with no clinical symptoms of intraocular inflammation. Moderate to mild conjunctival hyperemia was observed in a few dogs with LFA implants and Xalatan® drops. Central corneal thickness remained unchanged over the course of the study (PA5004: 602 ± 21 SEM μm baseline, 607 ± 16 SEM μm 24 wks; PA5108: 639 ± 9 SEM μm baseline, 648 ± 23 SEM μm 24 wks; PA5024: 613 ± 27 SEM μm baseline, 617 ± 18 SEM μm 24 wks). In 2 eyes, with longer implants, the implants were found to move and rub the corneal endothelium and induced focal corneal edema. Retinal thickness and optic nerve head measurements, electroretinograms, and visual performance did not reveal any treatment-related adverse effects.

**Conclusions:** Conclusions: Continuous IC LFA release via PolyActiva implants is a safe tool for long-term IOP control. The mechanical adverse effect on the cornea can be prevented by shortening the implants.

**Commercial Relationships:** Kristin Koehl; None; Christine Harman; None; Gabriel Stewart; None; Noah Wolinski; None; Taylor N. Norris; None; David Valade; PolyActiva (P); PolyActiva (E); PolyActiva (I); Andrew C. Donohue; PolyActiva (P); PolyActiva (E); PolyActiva (I); Igor Chekhman; PolyActiva (E); PolyActiva (I); John N. Lambert; PolyActiva (E); PolyActiva (I); Russell Tait; PolyActiva (S); PolyActiva (P); PolyActiva (E); PolyActiva (I); Andras M. Komaromy; PolyActiva (F).

**Program Number:** 4593 Poster Board Number: B0294

**Presentation Time:** 11:00 AM–12:45 PM

**Quantitative Ocular and Whole-Body Autoradiography Following a Single Topical Ocular Administration of Trabodenoson to Rabbits**

**Carrie C. Murray¹, Adam Brockman¹, David Albers², William K. McVicar³.** ¹Medical Affairs, Inotek Pharmaceuticals, Lexington, MA; ²Preclinical Development, Inotek Pharmaceuticals, Lexington, MA.

**Purpose:** To evaluate the ocular and systemic distribution and pharmacokinetics of trabodenoson in pigmented rabbits following a single topical ocular instillation.

**Methods:** A single drop of radiolabeled trabodenoson (¹³C-INO-8875) was instilled in the left eye (40 μl; 0.8 mg/eye) of Dutch-Belted rabbits (1.5-2 kg; age 5-6 mths). Animals were monitored daily, and blood, urine, and feces was collected 0-4 hrs, 4-8 hrs, 8-24 hrs, and at 24 hr intervals thereafter through 168 hours post-dose. Radioactivity levels in all samples were measured by liquid scintillation counters. Additionally, levels of parent compound as well as up to nine metabolites were quantified by radio-HPLC. Quantitative autoradiography was performed on whole body as well as on selected systemic and ocular tissues at various timepoints from 0.25 through 96 hrs post dose.

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**Purpose:** The neuro-protective properties of alpha 2-adrenergic agonists have been demonstrated in experimental studies. In this retrospective longitudinal study, we aimed to investigate visual field progression in glaucomatous eyes on mono-therapy with prostaglandin analogs (PA) and brimonidine eyedrops.

**Methods:** Glaucmatous eyes with 4 or more reliable 24-2 SITA-FAST (Humphrey® Field Analyzer) were included from the Kresge Eye Institute database. Reliability was considered less than 33% fixation losses, and false positive and false negative errors. In each eye, a linear regression was performed on mean deviation over follow-up time to obtain the rate of change (dB/year).

**Results:** A total of 99 eyes (65 patients) were included in the study while on brimonidine mono-therapy (36 eyes) and PA mono-therapy (63 eyes). The mean ± SD of follow-up time (yr) was 5.71 ± 4.59 and 5.07 ± 2.15 in the brimonidine and PA groups, respectively (p=0.15). The mean ± SD of number of visual field tests was 4.61 ± 1.05 and 5.02 ± 1.02 in the brimonidine and PA groups, respectively (p=0.06). The mean ± SD of MD (dB) was -4.60 ± 6.24 and -3.38 ± 3.86 in the brimonidine and PA groups, respectively (p=0.23). Between the two groups the only statistically significant patient demographic variables were race (67% and 84% African American in the brimonidine and PA groups respectively, p=0.05), and baseline visual acuity (19.2 ± 20 and 12.1 ± 12 LogMar in the brimonidine and PA groups respectively, p=0.03). The mean ± SD of average IOP (mm Hg) at baseline was 16.94 ± 4.32 and 16.06 ± 4.45 in the brimonidine and PA groups, respectively (p=0.34). There was no statistically significant difference between the two groups in MD progression rate (-.27 ± .73 dB/yr for brimonidine and -.05 ± .53 dB/yr for PA, p=0.09).

**Conclusions:** We found no statistically significant difference between brimonidine and PA mono-therapy in visual field progression over an average follow up time of 5.36 ± 1.79 years in eyes with mild to moderate glaucoma. This needs to be further investigated in prospective studies with larger sample size and in eyes with more severe glaucoma.

| Number of eyes (patients) | 99 (65) | 36 (27) | 63 (38) | --- |
| Age (years), mean ± SD | 62.72 ± 12.75, 61.85 ± 13.45, 62.84 ± 13.22 | 0.77** |
| Follow up time (year), mean ± SD | 5.36 ± 1.79, 5.71 ± 4.59, 5.07 ± 2.15 |
| Gender (%) | Male 27 (42), 14 (52), 13 (34) |
| Female 38 (58), 13 (48), 25 (66) |
| Race (%) | White 12 (18), 8 (30), 4 (11) |
| African-American 50 (77), 18 (67), 32 (84) |
| Other n/a 3 (5), 1 (3), 2 (5) |
| Diabetes (%) | Yes 23 (35), 8 (30), 15 (39) |
| No 42 (65), 19 (70), 23 (61) |
| Hypertension (%) | Yes 44 (68), 18 (67), 26 (68) |
| No 21 (32), 9 (33), 12 (32) |

**Table 1. Comparison of different study outcomes in the study groups.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient (95% CI)</th>
<th>P value</th>
<th>Coefficient (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline BCVA (LogMAR)</td>
<td>1.84 (-0.66,3.02)</td>
<td>0.05</td>
<td>-4.2 (-3.36,1.52)</td>
<td>0.67</td>
</tr>
<tr>
<td>Baseline IOP (mm Hg)</td>
<td>-0.02 (-0.10,0.02)</td>
<td>0.66</td>
<td>0.00 (-0.02,0.02)</td>
<td>0.95</td>
</tr>
<tr>
<td>Baseline CD (dB)</td>
<td>-.94 (-2.48,0.60)</td>
<td>0.24</td>
<td>-.28 (-1.52,0.96)</td>
<td>0.65</td>
</tr>
<tr>
<td>Baseline MD (dB)</td>
<td>-.04 (-.08,0)</td>
<td>0.06</td>
<td>0.03 (-.01,0.07)</td>
<td>0.14</td>
</tr>
<tr>
<td>Baseline PSD (dB)</td>
<td>-.08 (-.02,0.07)</td>
<td>0.24</td>
<td>-.12 (-0.24,0)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

**Table 2. Univariate regression analysis between progression rate (dB/year) and different study outcomes.**

**Commercial Relationships:** Monica Ray, None; Nariman Nassiri, None; Chae Sik Kim, None; Anju Goyal, None; Justin Tannir, None; Aman Shukairy, None; Mark S. Juzycz, None; Bret A. Hughes, None
**Purpose:** To evaluate the pharmacologic profile and distribution of CKLP1 following intravenous and topical eye administration.

**Methods:** CKLP1 and its active parent compound levcromakalim were evaluated in blood, plasma, aqueous humor, vitreous humor and selected tissues (brain, heart, kidney, liver, lung, muscle) by LC/MS/MS, following intravenous (IV; single injection, 0.25 mg/kg, n=6) and short (50 ul of a 10 mM solution, once daily for 8 days, n=12) or long term (50 ul of a 10 mM solution, once daily for 90 days, n=6) topical eye treatment in female Dutch belted pigmented rabbits. IOP in the topically treated animals was measured with a hand held rebound tonometer 3 times daily during the short treatment period and twice weekly for the long-term treatment study. Necropsy was performed on all animals and histology was assessed masked in ocular and non-ocular tissue samples.

**Results:** Following IV and topical administration, CKLP1 was rapidly converted into its active parent compound levcromakalim. In IV treated animals, CKLP1 and levcromakalim showed an average terminal half-life in plasma of 61.8 ± 55.2 min and 85.0 ± 37.3 min respectively. Mean maximum plasma concentration of levcromakalim was 10.6 ng/ml at 1h and 5.9 ng/ml by 8 h. In the short-term topical treatment group, the mean terminal half-life was 30 min for CKLP1 and 2 h for levcromakalim. Maximum plasma concentration of levcromakalim was 0.62 ng/ml at 4 h. Average concentration of levcromakalim was 0.15 ng/ml and 0.13 ng/ml in aqueous and vitreous humor respectively, while CKLP1 was below quantifiable levels. Long term topical treatment with CKLP1 resulted in significant IOP reduction (14.1 ± 1.0%, p<0.0001). No significant pathology was noted in any of the studied ocular and non-ocular tissues. Following 90 days of topical treatment, CKLP1 was detected in kidney (15.8 ng/ml, n=6) while levcromakalim was only detected in lung (1.6 ng/ml, n=6) and liver (5.9 ng/ml, n=4).

**Conclusions:** The produrg CKLP1 is readily converted to its active parent compound levcromakalim following intravenous and topical eye administration. Long-term treatment results in significant reduction in IOP with no apparent toxicity to ocular and non-ocular tissues.

**Commercial Relationships:** Uttio Roy Chowdhury, None; Rachel A. Kudugus, None; Tommy A. Rinkoski, None; Joel M. Reid, None; Michael P. Fautsch, None

**Support:** NIH grant EY21727, MN Partnership for Biotechnology and Medical Genomics, Research to Prevent Blindness, and Mayo Foundation

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**Program Number:** 4596 **Poster Board Number:** B0297

**Presentation Time:** 11:00 AM–12:45 PM

**Thrombin generation test of TLR4 antagonists compared to conventional anti-platelet drugs**

Indre Bielskus¹, Kevin Carey¹, Nicholas M. Pfahler³, Michael D. Miazga¹, Michael Giovingo¹, Paul A. Knepper¹, ³.

¹Ophthalmology and Visual Sciences, University of Illinois at Chicago, Chicago, IL; ²Ophthalmology, John H. Stroger, Jr. Hospital of Cook County, Chicago, IL; ³Ophthalmology, Northwestern University, Chicago, IL.

**Purpose:** Primary open-angle glaucoma (POAG) is a devastating disease of unknown etiology. Our lab has previously shown that both POAG and Alzheimer’s disease (AD) have a microvascular component. In this study, three inhibitors of the innate immune system receptor toll-4: resveratrol (R), quercetin (Q), and a µ-opioid antagonist (N) were compared to four commonly used anti-platelet drugs— aspirin, ibuprofen, dabigatran, and rivaroxaban. The purpose of this study was to determine the efficacy of RQN as a potential new anti-platelet therapy.

**Methods:** Whole blood was collected from control, POAG, and AD patients after obtaining informed consent and International Review Board approval. Following the method of Jobe et al (Blood;2015), washed platelets were isolated by centrifugation. Platelets (2x10⁶/ml) were activated with convulxin and thrombin, followed by addition of coagulation Factors V, X, and prothrombin, which exposed phosphotidylserine residues and converted prothrombin to thrombin. Chromogenic substrate S-2238 (Chromogenix) was used to determine thrombin generation as measured by a spectrophotometer at 405nm, and normalized to a standard curve. Base levels of thrombin generation were measured in controls, POAG, and AD. Three concentrations of RQN were tested, along with commonly used anti-platelet drugs — aspirin, ibuprofen, rivaroxaban, and dabigatran—in clinically used dosages.

**Results:** RQN at a 1µM concentration significantly reduced thrombin generation of washed platelets in controls (p=0.007), POAG (p=0.003), and Alzheimer’s disease (p=0.01). There was also significant reduction of thrombin generation at higher RQN concentrations and with use of other anti-platelet drugs, as shown in Table 1. RQN was as effective as conventional anti-platelet drugs in reducing thrombin generation.

**Conclusions:** RQN was effective in reducing thrombin generation in platelets. RQN blocks activation of the innate immune system and subsequent platelet activation through the three signaling pathways of the TLR4 receptor. In contrast, aspirin and ibuprofen act on the cyclooxygenase 2 pathway, dabigatran serves as a direct thrombin inhibitor, and rivaroxaban acts as a Factor X inhibitor. RQN prevents thrombin generation in a manner similar to conventional anti-platelet drugs; therefore, these results represent a new class of anti-platelet drugs to treat platelet-associated disease, namely POAG and AD.

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The clot may thicken in primary open angle glaucoma


Purpose: The cause of primary open angle glaucoma (POAG) is unknown. Recently, a multiple hit theory (Glaucoma Research and Clinical Advances: 2016 to 2018) posits three targets for POAG pathogenesis: microvascular insults to (1) ciliary body (CB) capillaries leading to trabecular meshwork cell death, (2) choroid capillaries leading to decreased cerebrospinal fluid production and an increased translaminar pressure gradient, and (3) lamina cribosa capillaries leading to optic nerve axon loss. Although the cause(s) of these microvascular insults are unknown, one key factor may be the role of vascular endothelial growth factor receptor (VEGFR2) in maintaining the fenestrated capillaries. The seminal work of D’Amore (IOVS:2012) demonstrated that VEGF neutralizing antibodies lead to damaged CB and CP. The purpose of the present study is to test the effects of the reported VEGF inhibitor β-amyloid (Aβ) 1-42 on platelet viscosity.

Methods: Blood samples were obtained from control (n=3) and POAG (n=3) patients after informed consent and International Review Board approval. Platelet rich plasma (PRP) was isolated by centrifugation at 300 x g for 30 min and challenged with graded amounts of thrombin (Sigma-Aldrich) to determine the lowest concentration of thrombin without a fibrin clot. PRP was treated with graded amounts of thrombin and Aβ 1-42 (Sigma) with and without the presence of 1µM amounts of selected toll-4 inhibitors—resveratrol (R), quercetin (Q), and a µ-opiate antagonist (N)—to prevent increases in viscosity and fibrin clot formation. PRP was subjected to a low shear rate of 50 seconds⁻¹ for 6 minutes at 37°C using a cone-plate rheometer (Brookfield).

Results: PRP treated with both Aβ and thrombin produced a seven-fold increase in viscosity when compared to thrombin or Aβ alone. This apparent synergism is present in both controls and POAG (p=0.002), as shown in Table 1. Treatment with a 1µM dose of RQN eliminated the Aβ-thrombin induced fibrin clots in both controls (p=0.04) and POAG (p=0.04).

Conclusions: Aβ 1-42 acts synergistically with thrombin to increase viscosity at a low shear rate. Notably, the toll-4 antagonists RQN prevented the Aβ synergism. The clinical implications of this finding include the increased likelihood of microvascular disease whenever shear rate is low, e.g., low blood pressure, sleep apnea, and migraine.
Table 1

| Commercial Relationships: | Michael Giovingo, None; | Kevin Carey, None; | Indre Bielskus, None; | Michael D. Miazga, None; | Nicholas M. Pfahler, None; | John R. Samples, None; | Paul A. Knepper, Testog, Inc. (P) |

Program Number: 4598 Poster Board Number: B0299
Presentation Time: 11:00 AM–12:45 PM

A novel method to reduce superactivated platelets in POAG and Alzheimer’s disease
Nicholas M. Pfahler1, Indre Bielskus1, Michael D. Miazga1, Kevin Carey1, Elani Kaufman1, Madalyn Mandich1, Michael Giovingo1, Paul A. Knepper2,3. 1Ophthalmology and Visual Sciences, University of Illinois at Chicago, Chicago, IL; 2Ophthalmology, John H. Stroger, Jr. Hospital of Cook County, Chicago, IL; 3Ophthalmology, Northwestern University, Chicago, IL.

Purpose: Superactivated platelets (SAPs) are increased in primary open-angle glaucoma (POAG) and Alzheimer’s disease (AD). SAPs are a prothrombogenic subpopulation of platelets involved in microvascular disease. Previously, we reported that a synergism of resveratrol (R), quercetin (Q), and a u-opiate antagonist (N) in vitro decreases SAPs in controls by inhibiting the three legs of toll-like receptor 4 (TLR4) signaling (ARVO, 2015). The purpose of this study was to evaluate pre-clinical TLR4 inhibitors in vitro as an anti-platelet therapy for POAG and AD using flow cytometry and to evaluate the in vivo efficacy of TLR4 inhibitors in mice using flow cytometry and capillary microscopy.

Methods: Whole blood was drawn by venipuncture after Institutional Review Board and informed consent from POAG (n=8), AD (n=6), and control (n=12) subjects. PRP was isolated, pre-treated with µM doses of RQN, and challenged with thrombin and convulxin. Fluorophore-conjugated antibodies were used to identify SAPs by flow cytometry: anti-CD41-PE identifies all platelets, anti-PAC-1-FITC identifies activated platelets, and streptavidin-APC identifies SAPs. For in vivo studies, mice were pre-treated with 1 µM RQN 3h and 1h before intraperitoneal injection of 15 U/ml thrombin. In one trial (n=10), whole blood was drawn from inferior vena cava in order to analyze SAPs by flow cytometry. In a second trial (n=10), footpad hemorrhages were counted by capillary microscopy. Paired t-tests were used for statistical analysis and the Chou-Talalay method was used to determine synergistic drug effects.

Results: SAPs were significantly increased in POAG (p≤0.001) and AD (p=0.0069) compared with controls. A 1 µM dose of RQN in vitro reduced SAPs from 28.9±6.4% to 13.4±4.5% (a decrease of 53.1%, p≤0.001) in controls, from 52.9±5.3% to 25.4±6.2% (-52.1%, p<0.001) in POAG, and from 50.6±6.8% to 34.6±8.9% (-31.6%, p=0.0036) in AD. See Figure 1. In mice, 1 µM RQN in vivo reduced SAPs from 74.8±1.6% to 48.8±5.9% (-34.79±5.9%, p=0.0018) and footpad hemorrhages from 31.9 to 12.3 /100 capillaries (-61.6%, p=0.012) compared with controls. RQN at 1 µM gave a Chou-Talalay combination index value of 0.015, suggesting a strong synergistic effect.

Conclusions: RQN decreases SAPs in POAG and AD subjects and decreases SAPs and footpad hemorrhages in thrombin-challenged mice. The RQN combination may represent a potential therapy for microvascular disease in POAG and AD.
The corneal epithelial barrier function is significantly disrupted along with tear secretion dynamics in patients on topical anti-glaucoma drugs for a prolonged time. This is indicative of ocular surface dysfunction, which not only reduces the success rate of filtering surgeries but is also associated with the loss of quality of life.

**Commercial Relationships:** Deepti P. Talele, None; ARUSHI GOYAL, None; RR Sudhir, None; Pavani Murthy, None; Prema Padmanabhan, None; Uday B. Kompella, None; Sangly P. Srinivas, None

**Support:** Obama-Singh Initiative award (PI-SP)

**Program Number:** 4600 Poster Board Number: B0301

**Presentation Time:** 11:00 AM–12:45 PM

**Artificial tear substitutes and ocular surface in glaucoma patients with chronic treatment**

*Methods:* Prospective, observational, multicentre, open label study. We enrolled 60 POAG patients treated with a preserved prostaglandins eye drops with OSD clinical signs detected by an Oxford score >8 after fluorescein/lissamine green staining (Oxford score grading 0-15). OSDI questionnaire, BUT, Schirmer test, fluorescein and lissamine green staining by Oxford score were performed at baseline (T0) and then repeated 7 days (T7) and 30 days (T30) after preservative free tear substitutes administration. At T0 patient were randomly assigned to a TID four week treatment with a cytoprotective tear substitute: Threalose/Hyaluronic acid solution (Group A) or Coenzyme Q10/vitamin E/Hypromellose solution (Group B). A third group received 0.9% saline solution TID for four week (Group C). Oxford Score reduction was considered the primary study endpoint.

**Results:** Oxford score and OSDI decreased in all groups vs baseline (p<0.001). In Oxford score a statistically significant difference (p<0.05) was observed only between group A and C (delta vs T0 - 4.8 vs -3.0 respectively). OSDI score decrease: group A (-19.3) and B (-16.0) > group C (-8.0)(p<0.05). No statistically significant difference was found between group A and C (delta vs T0 - 4.8 vs -3.0 respectively). OSDI score decrease: group A (-19.3) and B (-16.0) > group C (-8.0)(p<0.05). No statistically significant difference was found between group A and C (delta vs T0 - 4.8 vs -3.0 respectively). OSDI score decrease: group A (-19.3) and B (-16.0) > group C (-8.0)(p<0.05).

**Conclusions:** In OSD due to preserved anti-glaucoma treatment, cytoprotective tear substitutes (Threalose/Hyaluronic acid and Q10/vitE/Hypromellose) provide a better efficacy than 0.9% saline solution for sign and symptoms relief.
Commercial Relationships: Michele M. Jester, None; Michele Figus, None; Paolo Fogagnolo, None; Paolo Frezzotti, None; Francesco Oddone, None; Antonio Ferreras, None

Program Number: 4601 Poster Board Number: B0302
Presentation Time: 11:00 AM–12:45 PM

LOXL1 and Misfolded Protein Processing Pathways In XFS Glaucoma Cells
J Mario Wolosin1, Zheng Wang2, Stephanie Gillespie3, Shozer Dawn4, Robert Ritch1, Audrey M. Bernstein1. 1Ophthalmology, Eye and Ear Infirmary of Mount Sinai, New York, NY; 2Ophthalmology, Icahn School of Medicine at Mount Sinai, New York, NY.

Purpose: Tenon fibroblasts (TFs) from eyes with exfoliation syndrome (XFS) and glaucoma display autophagy impairment features similar to age-related neurodegenerative diseases (PLoS ONE 2016, 11: e0157404). The latter are frequently linked to high misfolding frequency in protein variants. Since XFS pathology is associated with two non-synonymous variants of LOXL1, we examined whether in XFS cells LOXL1 polypeptides are processed by misfolding pathways.

Methods: TF cultures were derived from XFS and primary open angle glaucoma (POAG, control) patient tissue. Intracellular protein aggregates were visualized with Proteostat™. Autophagy was accelerated by FBS removal (starvation). Co-IP was used to identify interaction between USP19 and LOXL1. Analytical methods included Western blot, immunostaining and electroporation.

Results: Proteostat staining demonstrated the presence of aggregated protein only in XFS cells. Aggregate presence in XFS TFs was also inferable from the much higher expression of clusterin, a protein expressed in response to aggregations (XFS/POAG = 1.9 fold, p=0.005). Total inhibition of autophagy by Spartin or Bafilomycin-A increased the LOXL1 concentration in XFS TF by 1.63±0.18, (p<0.01) and 1.41±0.01, (p<0.005), respectively. There was no comparable effect in POAG TFs, indicating that processing of LOXL1-containing aggregates by autophagy takes place only in XFS cells. Autophagy requires the close approximation of autophagosomes and lysosomes at the microtubule organizing center. The latter is held together by a Y shaped γ-tubulin complex (γ-TuSK) and anchored to the nuclear centrosomes via Ninein. Immunostaining for these proteins revealed that in XFS cells the complex is small and mislocalized compared to the well-developed, mature γ-TuSK seen in POAG cells. A USP19-dependent export mechanism for misfolded proteins has been recently described (Nat Cell Biol. 2016;18:765). Co-IP studies demonstrated association of USP19 with LOXL1 in the XFS conditioned medium but not in the POAG medium. Additionally, knockdown of USP19 (>80%) by siRNA significantly reduced the amount of LOXL1 exported, indicating that USP19 acts as a chaperone for LOXL1 in XFS cells.

Conclusions: Cellular pathways that handle misfolded proteins are actively clearing LOXL1 polypeptides in XFS cells.

Commercial Relationships: J Mario Wolosin, None; Zheng Wang, None; Stephanie Gillespie, None; Shozer Dawn, None; Robert Ritch, None; Audrey M. Bernstein, None

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Program Number: 4602 Poster Board Number: B0303
Presentation Time: 11:00 AM–12:45 PM

Evaluation of LOXL1 as a potential diagnostic and therapeutic target in exfoliation glaucoma

Purpose: Exfoliation glaucoma (XFG) is a common form of secondary glaucoma caused by progressive accumulation of insoluble elastic fiber-based microfibrillar deposits in the eye. XFG is the ocular manifestation of the age-related systemic disorder known as pseudoexfoliative syndrome (PXS); fibrillar deposits are found in various organs of PXS patients including the skin, heart, lungs, and brain. The disease is highly prevalent in the elderly in certain ethnic groups, suggesting the role of genetic and environmental factors. Its exact pathogenesis is not known, however, multiple single nucleotide polymorphisms in the lysyl oxidase-like 1 (LOXL-1) gene have been identified to correlate with PEX. LOXL1 belongs to the family of amine oxidases responsible for the maintenance and crosslinking of elastin and collagen fibers. LOXL-1 is a component of pseudoexfoliative material and its expression is upregulated in the early stages of the disease, therefore, targeting LOXL-1 may be a viable strategy to treat XFG.

Methods: In order to develop assays that could serve as tools for the diagnosis of XFG from human samples based on LOXL-1 activity and to evaluate the enzyme as a potential drug target, we expressed and purified human LOXL-1 from E. coli constructs. Enzyme activity was measured using colorimetry, fluorimetry, and luminometry, leveraging the enzyme’s ability to generate aldehydes and hydrogen peroxide. Small molecules were screened using differential scanning fluorimetry for possible interaction with LOXL-1.

Results: A chemiluminescence-based assay method was developed that can be used to quantify lysyl oxidase activity in biological samples. Using differential scanning fluorimetry, we identified small molecules that affect the stability of LOXL-1 and screened these for enzyme inhibition.

Conclusions: Our results highlight LOXL-1 as a potential target for identifying and treating XFG.

Commercial Relationships: Andras Varadi, None; Konstantin Petrukhin, None

Program Number: 4603 Poster Board Number: B0304
Presentation Time: 11:00 AM–12:45 PM

Inhibition of Transforming Growth Factor-β2 Signaling Prevents ECM Remodeling, Endoplasmic Reticulum Stress and Ocular Hypertension in Steroid-induced Glaucoma
Ramesh Kasetti1, Prabhavathi Maddinieni1, Pinkal Patel2, J Cameron Millar1, Tien Phan1, Charles C. Searby1, Abbot F. Clark2, Val Sheffield2, Gulab Zode3. 1North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, TX; 2Department of Pediatrics, University of Iowa, Iowa city, IA.

Purpose: Ocular hypertension is a serious side effect of glucocorticoid (GC) therapy. Abnormal accumulation of extracellular matrix (ECM) and chronic endoplasmic reticulum (ER) stress in the trabecular meshwork (TM) is associated with GC-induced ocular hypertension. In the present study, we examined the role of TGFβ2 signaling in dexamethasone (Dex)-induced ECM remodeling, ER stress and ocular hypertension.

Methods: Conscious IOP and outflow facility was measured in C57 mice treated with vehicle or Dex eye drops up to 7-weeks. TGFβ2 & fibromectin levels in the aqueous humor (AH) were analyzed by Western. Effect of inhibition of TGFβ signaling on Dex-induced ER stress and ECM accumulation was examined by Western blot, immunostaining & Smad reporter assay in TM cells treated with or
without TGFβ signaling inhibitors (SIS3 and LY364947). To further examine the role of TGF-β2 signaling, IOP, ECM and ER stress was examined in WT or Smad3−/− mice treated with DEX.

**Results:** Dexamethasone (Dex) mediated reduction of outflow facility and IOP elevation is associated with increased abnormal extracellular matrix (ECM) accumulation in the TM, inducing ER stress. Biochemical analysis of the aqueous humor samples from Dex-treated eyes revealed significantly increased bioactive form of transforming growth factor-β2 (TGF-β2), a major regulator of ECM in the TM. Dex treatment increased both precursor and bioactive form of TGF-β2 in the conditioned medium and activated TGF-β2-induced Smad signaling pathway in primary human TM cells as evident from increased phosphorylation of Smad3 and increased Smad luciferase activity. Inhibition of TGF-β2 signaling significantly reduced Dex-induced abnormal intracellular ECM accumulation and ER stress in human TM cells. Smad3−/− mice, which are required for TGF-β2 signaling, protected from Dex-induced ocular hypertension, ER stress and abnormal ECM accumulation further indicating the role of TGF-β2 signaling in GC-induced glaucoma. Interestingly, knock out of ER stress-induced transcriptional factors, ATF4 and CHOP prevented activation of TGF-β2 signaling and also reduced intracellular ECM accumulation in the TM, thus preventing Dex-induced ER stress and IOP elevation.

**Conclusions:** These studies indicate that Dex-induced TGF-β2 signaling is responsible for ECM remodeling, ER stress and elevation of IOP in GC-induced glaucoma.

**Commercial Relationships:** Ramesh Kasetti, None; Ericka Oglesby, None; J Cameron Millar, None; Cameron Phan, None; Charles C. Searby, None; Abbot F. Clark, None; Julie Schaub, None; Pinkal Patel, None; Gulab Zode, None; Ian Pitha, None; Harry A. Quigley, None

**Support:** NIH/NEI- EY 02120, K08EY024952, EY 01765 Glaucmia Research Foundation

**Program Number:** 4605 Poster Board Number: B0306

**Presentation Time:** 11:00 AM–12:45 PM

**Elevated hydrostatic pressure increases the sensitivity of optic nerve head astrocytes to an oxidative challenge**

**Purpose:** Primary open angle glaucoma (POAG) is often associated with elevated intraocular pressure (IOP), manifesting in a pathological triad of optic nerve head remodeling, damage to the optic nerve, and retinal ganglion cell loss. Optic nerve head astrocytes (ONHAs), the primary cell type in the optic nerve head, undergo significant pathological changes in POAG. Increased levels of oxidative stress, secondary to elevated IOP, are strongly implicated in the pathophysiology of POAG. Here, the cellular and molecular consequence of elevated hydrostatic pressure on cultured ONHAs responses to an oxidative challenge was investigated.

**Methods:** Primary adult rat ONHAs were exposed to ambient or elevated hydrostatic pressure (25-30 mm Hg above ambient pressure) for 16h using a custom-built cell culture pressure chamber. Cells were subsequently challenged with chemically-induced oxidative stress using tert-butylhydroperoxide (tBHP; 0-500 μM for 5h). In some cases, ONHA cultures were pre-treated with the antioxidant Trolox (100 μM). Cell viability was measured using plate reader-based MTT or lactate dehydrogenase (LDH) release assays; levels of oxidative stress were quantified using the fluorescent indicator, CellROX®.

**Results:** ONHA cultures exposed to elevated hydrostatic pressure exhibited a marked increase in the level of reactive oxygen species as quantified by CellROX® fluorescent staining, but did not significantly alter cell viability (n=3, p=0.65). Comparing to ambient control cultures, ONHA cultures exposed to elevated hydrostatic pressure exhibited a significant increase in sensitivity to a subsequent oxidative challenge [LD50 for tBHP were 179 ± 2 μM (ambient) vs. 84 ± 1 μM (elevated pressure); n=3, P<0.01 in the MTT assay and 145 ± 5 (ambient) vs. 67 ± 6 μM (elevated pressure); n=3, P<0.01 in the LDH assay]. By comparison, Trolox shifted the LD50 to a similar extent under both ambient (D = 50 ± 2 μM; n=3, P=0.01) and hydrostatic pressure (D = 48 ± 2 μM; n=3, P<0.01).

**Conclusions:** Short-term exposure to elevated hydrostatic pressure increases the levels of ROS in cultured ONHAs without affecting cell viability, but causing a significant increase in the sensitivity

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of ONHAs toward a subsequent oxidative challenge. These data suggest that even modest exposure to elevated IOP in POAG may significantly alter the oxidation response of ONHAs.

**Commercial Relationships:** Vidhya R. Rao, None; Alexandra D. Hegel, None; Jamie C. Floss, None; Jonathan Lautz, None; Vicki Husak, None; Evan B. Stubbs, None; Simon Kaja, None; Harry A. Quigley, University Chicago (SK), Dr. John P. and Therese E. Mulcahy Endowed Professorship in Ophthalmology (SK), Department of Veterans Affairs (SK); grant I21RX001593 to EBS

**Program Number:** 4606

**Presentation Time:** 11:00 AM–12:45 PM

**Mitochondrial movement and density in mouse ocular explant model after experimental glaucoma**

Elizabeth Cone-Kimball, Mary Ellen Pease, Julie Schaub, Ericka Oglesby, Ian Pitha, Cathy Nguyen, Harry A. Quigley. Ophthalmology, Johns Hopkins University, Wilmer Eye Institute, Baltimore, MD.

**Purpose:** To study retinal ganglion cell intra-axonal changes with IOP elevation in an explanted mouse globe—optic nerve.

**Methods:** Mice expressing cyan fluorescent protein in mitochondria were exposed to bead-induced IOP elevation for 1 or 3 days. The optic nerve head and nerve were then imaged by laser scanning microscopy in special chambers to measure mitochondrial size, density, % in motion, and speed (both anterograde and retrograde directions). The explant model provides stable intra-axonal structure and functional measures for 3 hours ex vivo.

**Results:** In data submitted for publication, chronic IOP elevation led to 13% shorter mitochondria length (fission) and 30% greater density immediately behind the globe (p=0.025). IOP increase to 30 mm Hg for 60 minutes reduced % mitochondria in motion by 50%. In the present extended experiments, we found that normal mean mitochondrial length (2.5 ± 1.67 µm) was reduced to 2.3 ± 1.43 µm after 3 days of prior IOP elevation (n.s.). While short term IOP increase produced greater mitochondrial density, longer IOP increase led to lower density: down 19.2% @ 1 day, down 32.3% @ 3 days (p=0.12, p<0.001, t-test). Mean mitochondria speed was normally 0.19 µm/s anterograde and 0.32 µm/s retrograde (p=0.001, t-test), with 3X more moving anterograde (p=0.05, t-test) and moving longer distances in anterograde between pauses (8.22 µm vs 7.32 µm, p=0.5, t-test). In controls, 6.3% of mitochondria were moving in either direction at a given time, but that decreased to 1.2% after 1 day of IOP elevation (1 of 173).

**Conclusions:** Changes in mitochondrial structure, density and speed may underlie or represent important events in glaucomatous axonopathy. Optic nerve explants provide real time measurement of the earliest events in experimental glaucoma as well as quantitative outcomes for neuroprotection therapy development.

**Figure 1. A:** Longitudinal image of Optic nerve head showing mitochondria expression in control explant. B: 3 day glaucoma explant showing mitochondrial transport block with clumping (arrow head, bar = 10 µm).

**Commercial Relationships:** Elizabeth Cone-Kimball, None; Mary Ellen Pease, None; Julie Schaub, None; Ericka Oglesby, None; Ian Pitha, None; Cathy Nguyen, None; Harry A. Quigley, None

**Support:** NH EY02120 and NH EY01765

**Program Number:** 4607

**Presentation Time:** 11:00 AM–12:45 PM

**Agnostic β2-adrenergic receptor autoantibody positivity in progressive glaucoma suspects**


**Purpose:** Agnostic autoantibodies (AAB) against G protein-coupled receptors (GPCR) have been found in several diseases including asthma and arterial hypertension. β2-adrenergic receptors (β2-AR) are expressed in trabecular meshwork and ciliary body, being potentially involved in the regulation of aqueous humor dynamics. Agnostic β2-adrenergic receptor autoantibodies (β2-AABs) have been detected in ocular hypertension (OHT) and glaucoma patients, yet not in healthy controls. The aim of this study was to compare the rate of perimetric progression in glaucoma suspects in the presence and absence of β2-AAB.

**Methods:** 43 glaucoma suspects were included in the study. All patients showed an increased intraocular pressure and in part a glaucomatous optic disc alteration without any perimetric defects. Annually, all patients underwent complete ophthalmological examinations including visual field testing (Octopus G1). Serum samples were analyzed for the presence of β2-AAB. Longitudinal perimetric analyses were performed over a follow-up period of 18 years. Perimetric progression was defined as a MD>2.8 and ≥3 adjacent test points on the pattern deviation map with a probability of <5% or ≥2 adjacent test points on the pattern deviation map with a probability of <1%, confirmed at least once. The protocol was approved by the local Ethics Committee (no. 3457) and the study was registered.

**Results:** β2-AAB were detected in 31 of 43 glaucoma suspects (72%), whereas 12 patients showed no β2-AAB (28%). A perimetric progression was seen in 6 of 62 eyes of the β2-AAB positive patients (10%). However a perimetric progression was only seen in 1 of 24 eyes of patients without β2-AAB (4%). β2-AAB positivity was found in 86% of all glaucoma suspects, which showed a progressive visual field.

**Conclusions:** In this pilot study β2-AAB were detected in a large number of glaucoma suspects. In addition the presence of β2-AAB seemed to be associated with the development of visual field defects. First we plan to confirm this relationship in a larger cohort. Secondly we wish to investigate the potential mechanism, by which β2-AAB impair optic nerve head function.
Increased expression of P2X, receptor induced by DHPG in retinal ganglion cells
Min Ji, Huaijin Guan. Ophthalmology, Affiliated Hospital of Nantong University, Nantong, China.

**Purpose:** To determine the direct effect and the possible mechanism of DHPG, an mGluR I agonist, to retinal ganglion cells.

**Methods:** DHPG was intravitreal injected into the left eyes of rats. The apoptosis of RGCs was determined by Retrograde Labeling and Counting of RGCs and TUNEL stain in cultured RGCs. Expression of mGluR I (mGluR1,mGluR5) and P2X7R protein was determined by immunohistochemistry and Western blot analysis. Mixed culture of retinal neurons were prepared for the detection of the expression of P2X7R and the ATP current. Whole-cell patch clamp was introduced to detect the change of the ATP current of RGCs.

**Results:** The number of RGCs significantly decreased in retina of rats with DHPG intravitreal injection and partly blocked by BBG, the antagonist of P2X7 receptor. TUNEL positive cells increased in cultured RGCs with the presence of DHPG and partly blocked by BBG. Our previous study showed the activation might cause the apoptosis of RGCs. P2X7 receptor, mGluR1 and mGluR5 were all expressed in the ganglion cell layer(GCL) in normal retinal slice and in cultured RGCs. DHPG could increase the expression of P2X7R both in vivo and in vitro, and both MPMQ (the mGluR1 antagonist) and MPEP (the mGluR5 antagonist) could inhibit the effects. In cultured RGCs, the ATP current did not change with DHPG perfusion, while the ATP current increased with the presence of DHPG for 2 days in the culture solution. The increased ATP current levels could be inhibited both by MPEP and MPMQ.

**Conclusions:** These data demonstrated that DHPG increased the expression of P2X7 receptor through both mGluR1 and mGluR5, and finally cause the apoptosis of RGCs.

**Commercial Relationships:** Min Ji, None; Huaijin Guan, None
**Support:** National Natural Science Foundation of China (81200680; 81670852)
Hg (p<0.01) at 2-4 hours after dosing. Irritation scores were typically only trace (+0.5) hyperemia (Draize scale 0 – 3) lasting 4-8 hours. Addition of distal nitrogen atom produced compounds with maximal efficacy. Testing in in vitro stability assays revealed that resistance to racemization and other forms of in vivo degradation, and revealed that AR-13917 possessed the correct stereochemistry for further studies.

**Conclusions:** Compounds containing cyclopropyl acid amides produced significant reductions in IOP with an extended duration of action. Removal of sulphydryl activity preserved efficacy while improving tolerability in normotensive rabbits and monkeys. This class of Rho kinase inhibitors warrants further in vitro and in vivo studies.

**Commercial Relationships:** Mitchell A. deLong, Aerie Pharmaceuticals (E); Jill Sturdivant, Aerie Pharmaceuticals (E); Cynthia Lichorowic, Aerie Pharmaceuticals (E); Cheng-Wen Lin, Aerie Pharmaceuticals (E); Casey Kopezynski, Aerie Pharmaceuticals (E)

**Program Number:** 4611 Poster Board Number: B0312  
**Presentation Time:** 11:00 AM–12:45 PM

**Optimization of transgene expression in the trabecular meshwork (TM) by comparing capsid mutant AA V2 vectors**

Christine Harman,1 Kristin Koehl,1 Annie Oh1,2, Vince A. Chiodo,1 Sanford L. Boye,1 William W. Hauswirth,1 Shannon E. Boye,1, Andras M. Komaromy,1,2  
1College of Veterinary Medicine, Michigan State University, East Lansing, MI; 2College of Veterinary Medicine, North Carolina State University, Raleigh, NC;  

**Purpose:** We have previously reported successful targeting of GFP expression to the rodent TM with capsid-mutant AA V2. The purpose of our study was to optimize targeting of the canine TM with AA V2 by altering capsid mutations, pupil size, and route of intraocular vector administration.

**Methods:** 18 eyes of 11 adult, wild-type dogs were treated by intraocular injections of AA V2-GFP with a small chicken beta actin promoter. TM fluorescence was evaluated by gonioscopy and immunohistochemistry (IHC). First, we compared 3 capsid mutants at 2 doses (10⁹ and 10¹⁰ vg) following intracameral (IC) injection: single (Y444F), triple (Y444, 500, 730F), and quadruple (Y444, 500, 730F + T491V) capsid amino acid changes. Once we selected AA V2-Y444F as the benchmark vector, we compared doses over a range of 3 log units (10⁻⁹ to 10⁻⁸ vg), and evaluated the effect of pupil size at the time of IC injection (latanoprost-induced miosis vs. atropine-induced mydriasis) on TM transgene expression. Finally, we compared IC vs. intravitreal (IVit) vector administration. The dogs were monitored for up to 4.6 months after treatment. GFP expression within the ICA was measured by IHC using ImageJ (% GFP-stained area within iridocorneal angle in a 4x low-power field).

**Results:** Of the 3 capsid mutant vectors only AA V2(Y444F) resulted in detectable TM GFP expression and was used as the benchmark vector in subsequent experiments. While at the 10⁹ vg dose GFP fluorescence was barely detectable (0.01% area), the area of transgene expression was much larger (0.35-0.4%) with 10¹⁰ and 10¹¹ vg, respectively. GFP fluorescence was most robust with 10¹¹ vg (4.1% area, including both TM and uveoscleral outflow pathway) with clinically detectable fluorescence by gonioscopy. The highest vector dose also resulted in uveitis at 5 weeks post injection, a likely adverse reaction to the expressed GFP. There were no differences in transgene expression between the 4 quadrants. The extent of GFP expression within the TM was only minimally affected by the pupil size at the time of IC vector injection, with the mydriatic pupil resulting in lower fluorescence (2.8 vs. 4.8%). Both IC and IVit vector administration resulted in comparable TM GFP expression; levels were slightly higher with IC injection (4.1 vs. 2.8%).

**Conclusions:** IC injection of AA V2(Y444F) at a dose of 10¹¹ vg resulted in the most robust transduction of the canine TM.

**Commercial Relationships:** Christine Harman, None; Kristin Koehl, None; Annie Oh, None; Vince A. Chiodo, None; Sanford L. Boye, None; William W. Hauswirth, Bionic Sight (I), AGTC (C), AGTC (I); Shannon E. Boye, None; Andras M. Komaromy, None

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**Program Number:** 4612 Poster Board Number: B0313  
**Presentation Time:** 11:00 AM–12:45 PM

**Opioid Effects on Ischemic Stress in Human Trabecular Meshwork Cells**

Karen R. Russell Randall,1 Keiwana Glover,1 An Zhou1, Pharmacol & Toxicology, Morehouse School of Medicine, Atlanta, GA; 1Neurobiology/Neuroscience Institute, Morehouse School of Medicine, Atlanta, GA.

**Purpose:** Damage to trabecular meshwork (TM) cells seen in glaucoma, is mainly the result of oxidative stress, and causes high intraocular pressure, and ultimately damage to the optic nerve. Reducing damage to the TM is therefore important in preventing ocular hypertension and glaucoma. We hypothesize that opioids will have protective effects on normal human TM (NTM-5) cells exposed to an experimental model of ischemic stress, known to trigger oxidative responses and lead to cell death.

**Methods:** Immunochemical techniques using antibodies directed against kappa and delta opioid receptors (KOR, DOR) were utilized to determine receptor localization to NTM-5 cells. Receptor protein expression was determined by Western blot. An oxygen/glucose deprivation (OGD) model was used to simulate ischemic conditions. Cytotoxicity and cell viability were evaluated via lactate dehydrogenase (LDH) assay and Guava ViaCount system, respectively. Cells were treated with spirodoline (SPR; 0.1 - 10 mM), a highly selective KOR agonist, during and following OGD. Western blot was used to determine the effects of SPR on the apoptotic biomarkers caspases 3 and 9, as well as on the antioxidant, superoxide dismutase (SOD1). PBS controls were included with all experiments. Statistical significance (p<0.05) was obtained and evaluated using analysis of variance (ANOVA) followed by the Tukey-Kramer and Holm-Sidak multiple comparisons test.

**Results:** KORS and DORS were found localized to NTM-5 cells, and protein expression of these receptors was confirmed by Western blot. When compared to controls, NTM-5 cells pre-treated with SPR (24 hrs.), caused concentration-dependent decreases in cytotoxicity (p<0.001). Our data also showed that the protective effects of SPR was more evident with the inclusion of the drug during the OGD process, as well as during the recovery period (p<0.01). Cells pre-treated with SPR (10 µM) also showed increased cell viability when compared to control experiments (p<0.01). Pre-treatment with SPR also caused a reduction in the protein expression of caspases 3 and 9, and an increase in the protein expression of the antioxidant SOD.

**Conclusions:** This data provides evidence that a selective KOR agonist reduces apoptotic cell death and protects normal trabecular meshwork cells undergoing stress. Protecting the TM is important in preventing ocular hypertension, and opioids may have a very important role in this process.

**Commercial Relationships:** Karen R. Russell Randall, None; Keiwana Glover, None; An Zhou, None

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**Program Number:** 4613 Poster Board Number: B0314
**Presentation Time:** 11:00 AM–12:45 PM
**Role of Statins in the Development and Progression of Open-Angle Glaucoma**

*Nidhi Talwar, Joshua D. Stein.* Kellogg Eye Center, University of Michigan, Ann Arbor, MI.

**Purpose:** There is growing evidence that statins may protect against the development or progression of open-angle glaucoma (OAG). In order to further our understanding of this potential protective effect, we sought to determine whether certain types or daily dosages of statins provide greater protection against OAG relative to others.

**Methods:** Healthcare claims data from enrollees in a large US managed care network during 2001-2009 were used to identify all persons aged ≥60 years who had been diagnosed with hyperlipidemia. We excluded those with pre-existing OAG in a 2-year lookback period. Multivariable Cox regression was used to assess the potential association between statins and OAG, accounting for baseline low-density lipoprotein (LDL) level, use of other cholesterol-lowering agents, and other factors. Additional models were used to compare the effect of different types of statins to determine whether one type is more protective than others and whether the protective effect on OAG varied among exclusive users of 10, 20, 40, and 80mg daily dosages of statins.

**Results:** Among the 25420 eligible enrollees, 15898 persons (62.5%) had ≥1 record of statin use and 1218 (4.8%) developed OAG. After adjustment for confounding factors, persons taking statins continuously for 2 years had a 21% reduced risk of OAG compared to nonusers (adjusted HR=0.79, 95% CI=0.66-0.96, p<0.02). There was no statistically significant difference in the hazard of OAG among exclusive daily users of 10mg (HR=0.82, CI=0.61-1.11), 20mg (HR=0.92, CI=0.69-1.23), or 80mg (HR=1.03, CI=0.59-1.80), compared with daily users of 40mg statins. Compared to atorvastatin, there was no significant difference in hazard of OAG from taking lovastatin (HR=1.09, CI=0.71-1.68), cerivastatin (HR=0.61, CI=0.09-4.41), rosuvastatin (HR=0.83, CI=0.48-1.44), fluvastatin (HR=0.89, CI=0.39-2.02), pravastatin (HR=1.29, CI=0.93-1.79), and simvastatin (HR=1.03, CI=0.83-1.29).

**Conclusions:** Among persons with hyperlipidemia, after accounting for baseline LDL levels, those taking statins had a 21% reduced risk of OAG compared to nonusers. No other type of statin had a greater protective effect against OAG compared to atorvastatin, a common generic statin. There was no greater protective effect among users of 80mg a day relative to others taking 40mg a day. These findings will be useful to guide researchers as to what type and daily dosage of statins to use in clinical trials.

**Commercial Relationships:** Nidhi Talwar, None; Joshua D. Stein, None

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**Program Number:** 4614 Poster Board Number: B0315
**Presentation Time:** 11:00 AM–12:45 PM
**Medical Marijuana and Glaucoma: The United States Experience**

*Denise A. Valenti,* CEO/President, IMMAD, Quincy, MA.

**Purpose:** There are twenty-eight states that allow for the use of medicinal plant marijuana and the majority have glaucoma as an approved pathology for which marijuana can be used. These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to http://iovs.arvojournals.org/ to access the versions of record.

**Methods:** Each state was sent an email survey with questions specific to the use of marijuana as the diagnosis for certification to obtain or grow medicinal marijuana. Each survey was followed up by either additional email communications or a telephone call.

**Results:** Marijuana is being used as a treatment for glaucoma and there are indications that marijuana is being used to replace conventional pharmaceutical treatments, but this may be minimal. Data outside this project and published in peer reviewed journals provide additional evidence to support this conclusion. Further there is evidence in peer reviewed journals that those states that have medicinal marijuana have had behavior changes on the part of clinicians and patients that have resulted in an increased awareness of the need for glaucoma related evaluations. But based on the survey results, there are patients using marijuana to treat glaucoma and this may be in the absence of other eye care. There remains little to no education provided to a patient with glaucoma when seeking to use marijuana to treat the disease. Nor is there any indication that there is appropriate monitoring of the pressure in the eye or progression of the functional and structural changes that could lead to blindness when patients are treating the glaucoma with marijuana, in absence of other traditional glaucoma therapies.

**Conclusions:** More education to the general public is necessary regarding the use of marijuana as treatment for glaucoma. We need better surveillance tools regarding the use of medical marijuana and specifically marijuana to treat glaucoma. Patients electing to use marijuana as the only treatment for glaucoma are at risk for vision loss and blindness primarily because there may be an absence of adequate medical monitoring, follow up and guidance.

**Commercial Relationships:** Denise A. Valenti, IMMAD (I)

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**Program Number:** 4615 Poster Board Number: B0316
**Presentation Time:** 11:00 AM–12:45 PM
**A Pilot Study on the Effect of Alternate Nostril Breathing and Foot Reflexology on Intraocular Pressure in Ocular Hypertension**

*Jui Pandya, Martin Schardt, Daohai Yu, Jeffrey D. Henderer.* Ophthalmology, Temple University, Philadelphia, PA.

**Purpose:** Complementary and alternative methods (CAM) of intraocular pressure (IOP) treatment are often used by glaucoma patients. Alternate Nostril Breathing (ANB) and Foot Reflexology (FR), are two such treatments. However, peer-reviewed articles on the subject are limited. We conducted a pilot study to investigate the effects of ANB and FR to lower IOP in patients with ocular hypertension.

**Methods:** Eleven patients were recruited from Temple Ophthalmology between 2014 and 2016. Patients had ocular hypertension and were between the ages of 48-74. Patients were excluded if they currently performed ANB or FR, were unable to perform the task, had previous eye surgery or laser, were receiving other CAM for ocular hypertension, or were unable to complete a washout period. After a 30-day drug washout, patients completed either ANB or FR. After instruction, the assigned task was performed for 5 minutes. FR was done on a commercially available foot reflexology board (JAPAN TSUBO HH-700) focused at the base of the second and third toes of both feet. Two weeks later, patients completed the alternate task. IOP was measured before the task, immediately after the task and then every 30 minutes for two hours. Sign rank tests were used to evaluate IOP changes within and between each group.

**Results:** Baseline IOP was 25.86 ± 3.19mmHg for ANB and 25.41 ± 3.54mmHg for FR (N= 22 eyes). There was a significant decrease in IOP at 30 min post task for both ANB and FR with IOP decrease of 1.98 ± 1.70mmHg for ANB and 3.59 ± 1.99mmHg for FR (both p<0.0001). There was also a significant IOP lowering effect at 60 min...
post task with an IOP decrease of 2.39 ± 2.05mmHg for ANB and 3.86 ± 1.89mmHg for FR (both p<0.0001). The decrease in IOP at 90 and 120 min post task was less but remained statistically significant (p<0.0001). IOP decreased significantly more in FR group than ANB group for the 30 and 60 min time intervals (p<0.01) but there was no significant difference at 90 and 120 min (p>0.40).

**Conclusions:** Both foot reflexology and alternate nostril breathing had an IOP lowering effect in these 11 patients with foot reflexology having the greater IOP lowering effect for up to 60 min. Further studies will be necessary to determine the optimal treatment parameters in the hope that this form of CAM may be an additional intraocular pressure lowering option.

**Commercial Relationships:** Jui Pandya; Martin Schardt, None; Daohai Yu, None; Jeffrey D. Henderer, None

**Clinical Trial:** 02993445