ARVO 2015 Annual Meeting Abstracts

468 Aqueous dynamics, IOP, blood flow, clinical studies
Wednesday, May 06, 2015 3:45 PM–5:30 PM
Exhibit Hall Poster Session
Program #/Board # Range: 4848–4870/A0001–A0023
Organizing Section: Physiology/Pharmacology

Program Number: 4848 Poster Board Number: A0001
Presentation Time: 3:45 PM–5:30 PM
Identification of a highly efficacious, ultra-long acting ocular hypotensive agent in non-human primates
Carol B. Toris¹, Robert Coleman¹, Shan Fan², Amanda J. Woodroofe², David F. Woodward¹. Ophthalmology, Case Western Reserve University, Cleveland, OH; ²Ophthalmology, University of Nebraska Medical Center, Omaha, NE; ³Allergan, Irvine, CA; ⁴Asterand, Royston, United Kingdom.

Purpose: In a program to discover drugs that are meaningfully superior to currently available anti-glaucoma drugs in terms of efficacy, potency, and duration of action, the isopropyl ester of a novel structurally non-prostanoid EP, agonist, PGN9856 (PGN9856-I), was studied in unilateral ocular hypertensive monkeys.

Methods: Measurements included intraocular pressure (IOP), aqueous flow, fluorophotometric outflow facility, tonographic outflow facility, outflow facility by needle method, central cornea thickness and anterior chamber depth. Uveoscleral outflow was calculated by the Goldmann Equation. Twenty-four hours before measurements commenced animals were dosed once (25ul drop in each eye) with vehicle or drug in a random crossover design. Results with vehicle and drug were compared by two-tailed paired t-tests.

Results: IOP studies: PGN9856-I reduced IOP in ocular hypertensive eyes over a 0.0001% - 0.01% dose-range. After a single 0.006% dose, the maximal IOP reduction was reached at 48 hours, IOP being reduced to approximately half that of the ocular normotensive contralateral eye. Five days after this dose of PGN 9856-I, the IOP of the treated hypertensive eyes had recovered only to the level of the normotensive controls. The IOP of PGN9856-I treated hypertensive eyes did not return to baseline values until after approximately two weeks. While active, such profound effects in terms of either efficacy or duration of action were not seen with the free acid of this compound, PGN9856. No drug-related side effects were observed.

Aqueous humor dynamics study: Compared with vehicle treatment, IOP was reduced at 7, 24 and 26.5 hours after a single dose of 0.0003% PGN9856-I (p<0.0001). Aqueous flow decreased by 20% (p=0.02) and uveoscleral outflow doubled (p<0.03). Outflow facility was not changed when measured three different ways.

Conclusions: PGN 9856-I is an exceptionally potent, efficacious, and long-acting ocular hypotensive compound. It lowers IOP by a dual mechanism on aqueous humor inflow and uveoscleral outflow.

Commercial Relationships: Carol B. Toris, Allergan (F), Bausch and Lomb (F), Nicox (F); Robert Coleman, Allergan (F), Astereand (F); Shan Fan, None; Amanda J. Woodroofe, Asterand (E); David F. Woodward, Allergan (E)
Support: Allergan

Program Number: 4849 Poster Board Number: A0002
Presentation Time: 3:45 PM–5:30 PM
Aqueous humour outflow physiology in NOS3 knockout mice
Yuan Lei¹, Xuejin Zhang², Jihong Wu³, Xing-Huai Sun³. ¹Eye and ENT hospital of Fudan University, Shanghai, China; ²Key Laboratory of Myopia, Ministry of Health, Fudan University, Shanghai, China.

Purpose: To investigate the role of endothelial nitric oxide synthase (NOS) on conventional outflow function using NOS3 knockout (KO) mice.

Methods: IOP was measured in both NOS3 KO and wild type (WT) mice by rebound tonometry. Outflow facility was measured by perfusing enucleated mouse eyes (NOS3 KO versus WT) at multiple pressure steps between 8 and 30 mmHg. A subset of eyes embedded in paraffin was sectioned and stained using haemotoxylin and eosin (H&E) for histology. Mock aqueous humour or mock aqueous humour + the nitric oxide (NO) donors nitroprusside dihydrate (SNP, 1 uM) or S-Nitroso-N-Acetyl-D,L-Penicillamine (SNAP, 100 uM) was perfused into enucleated eyes. SNP (4x2ul drops, total dose 160 ug) and SNAP (4x2ul drops, total dose 160 ug) was administered topically at 0, 1, 2, and 3 hours while the contralateral eyes served as vehicle controls. IOP was measured in both eyes before drug treatment and 1 hour after the last drug treatment.

Results: IOP was higher (18.2 ± 3.9 vs. 13.9 ± 2.5 mm Hg; mean ± SD, P<0.05) in KO mice, and pressure-dependent conventional drainage was significantly lower (0.0058 ± 0.0010 uL/min/mmHg, mean ± SEM, n=21) compared with WT mice (0.0087 ± 0.0013, uL/min/mmHg, n=23, p<0.05). No obvious morphological difference in iridocorneal angle tissues was observed in H&E stained mouse eye sections. SNP and SNAP significantly increased pressure-dependent drainage in KO animals from 0.0062 ± 0.0009 to 0.01465 ± 0.0050 uL/min/mmHg (mean ± SEM, n=12, p<0.05), and from 0.0067 ± 0.0015 to 0.0223 ± 0.0044 uL/min/mmHg (mean ± SEM, n=12, p<0.05), respectively. In WT mice, SNP and SNAP caused a significant increase in pressure dependent drainage (n=12, p<0.05) to a similar degree to KO mice. Topical application of SNP significantly reduced IOP in WT and KO mice by 31% and 30% respectively (n=12, p<0.05), but SNAP did not change IOP significantly (n=12).

Conclusions: NOS3 KO mice have elevated IOP which is likely the result of reduced pressure-dependent drainage. These findings are consistent with human data showing polymorphisms in NOS3 gene associate with ocular hypertension and the development of glaucoma.

Commercial Relationships: Yuan Lei, None; Xuejin Zhang, None; Jihong Wu, None; Xing-Huai Sun, None
Support: National Science Foundation China (81100662, 81371015), Shanghai Municipal Health Bureau Young Outstanding Scientist Program (XYQ2013083), 211 Project of Fudan University (EHF158351), Scientific Research Foundation for the Returned Overseas Chinese Scholars (State Education Ministry)

Program Number: 4850 Poster Board Number: A0003
Presentation Time: 3:45 PM–5:30 PM
Variations in Active Areas of Aqueous Humor Outflow Through the Trabecular Outflow Pathway
Elliott D. Cha, Jia Xu, Haiyan Gong. Ophthalmology, Boston University School of Medicine, Boston, MA.

Purpose: Previous studies in human eyes suggest that outflow through the trabecular meshwork (TM) and inner wall (IW) of Schlemm’s canal is segmental. Whether these patterns are conserved in distal outflow pathways remains to be determined. This study aims to evaluate variations of active outflow along the trabecular outflow pathway in three distinct regions: TM, IW and episcleral veins (EPV); and to develop a simple imaging method to assess active outflow areas on a whole eye scale.

Methods: Six normal human eyes were perfused for 30 minutes at 15mmHg to establish a baseline outflow facility. The anterior chamber of each eye was exchanged (5mL) and perfused with a fixed volume of fluorescent microspheres (200uL) to label outflow patterns. All eyes were perfusion fixed. Anterior segments were dissected and the TM and EPVs were imaged globally. Global images were analyzed for tracer distribution and intensity using an ordinal scale and effective filtration area (EFA) was measured. Anterior segments were dissected into a minimum of 16 radial

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wedges. Frontal sections were cut from each wedge, imaged for tracer distribution along the IW by confocal microscopy, as well as both EFA and number of collector channels (CC) analyzed. Student’s t-test and correlation analysis was performed.

**Results:** Average baseline outflow facility was 0.18±0.08 μL/min/mmHg. Active outflow in the TM showed a less segmented pattern and a significantly higher EFA (84.06±5.89%) than both the IW (35.32±5.03%) and EPVs (32.31±3.90%; p<0.05). Comparisons of IW and EPV EFA revealed differences on an individual wedge basis, but a similar percentage of EFA was found on a whole eye basis (p=0.05). Quadrant analysis of tracer distributions in EPVs revealed a preferential flow to both the nasal and inferior quadrants. No preference was found in the TM. Both IW and EPV EFA was significantly higher when one or more CCs were observed compared to no CCs (p<0.05; p<0.01).

**Conclusions:** Percentage of EFA was found to be similar in both IW and EPVs. Therefore, determining EFA in EPV could be used as a simple and useful tool to determine the area of active outflow on a whole eye scale, which may have clinical implications for future management of glaucoma.

**Commercial Relationships:** Elliott D. Cha, None; Jia Xu, None; Haiyan Gong, None

**Support:** NIH EY022634, The Massachusetts Lions Eye Research Fund

**Program Number:** 4851 **Poster Board Number:** A0004

**Presentation Time:** 3:45 PM–5:30 PM

**Comparison of Aqueous Outflow Facility Measurement by Pneumatonography and Digital Schiøtz Tonography**

**Arash Kazemi**, Jay W. McLaren, Sayoko E. Moroi, Carol B. Toris, Shuai-Chun Lin, Arthur J. Sit. Ophthalmology, Mayo Clinic Rochester, Rochester, MN; Ophthalmology, University of Michigan, Ann Arbor, MI; Ophthalmology, Case Western Reserve University, Cleveland, OH.

**Purpose:** Electronic Schiøtz tonography was once the standard method for measuring outflow facility but it is no longer commercially available. This method has been replaced by a commercial pneumatometer with a tonography option. In this study we compared outflow facility measured by pneumatonography to outflow facility measured by digital Schiøtz tonography.

**Methods:** Fifty eyes from 25 healthy adult subjects (age 41-67 years, mean 50 years) were examined as an ancillary study to an ongoing study. Methods: Outflow facility was then measured by pneumatonography (10-gm weight, 2 minutes) and 45 minutes later by custom digital Schiøtz tonography (5.5-gm weight, 4 minutes) in the supine position. The weight, 2 minutes) and 45 minutes later by custom digital Schiøtz tonography (5.5-gm weight, 4 minutes) and a significantly higher EFA (84.06±5.89%) than both the IW (35.32±5.03%) and EPVs (32.31±3.90%; p<0.05). Comparisons of IW and EPV EFA revealed differences on an individual wedge basis, but a similar percentage of EFA was found on a whole eye basis (p=0.05). Quadrant analysis of tracer distributions in EPVs revealed a preferential flow to both the nasal and inferior quadrants. No preference was found in the TM. Both IW and EPV EFA was significantly higher when one or more CCs were observed compared to no CCs (p<0.05; p<0.01).

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**Commercial Relationships:** Elliott D. Cha, None; Jia Xu, None; Haiyan Gong, None

**Support:** NIH EY022634, The Massachusetts Lions Eye Research Fund

**Program Number:** 4852 **Poster Board Number:** A0005

**Presentation Time:** 3:45 PM–5:30 PM

**Trabecular Meshwork Exosomes: Fibronectin Binding and ECM homeostasis**

W M. Dismuke, W D. Stamer. Ophthalmology, Duke University, Durham, NC.

**Purpose:** Primary human trabecular meshwork (hTM) cells release extracellular nanovesicles called exosomes. While their function(s) in conventional outflow biology is unknown, hTM exosomes bind soluble fibronectin (Fn) and other extracellular matrix (ECM) components. We hypothesize that TM cells utilize this feature for efficient uptake of digested ECM.

**Methods:** Exosomes were purified from media conditioned by mature hTM cell monolayers via serial ultracentrifugation. Protein composition was determined by LC-MS/MS and western blot. Vesicle size was measured by nanoparticle tracking analysis (NTA). Dipeptidyl peptidase IV (DPPIV) activity was assessed by fluorogenic substrate cleavage. Fn conformation was monitored by detergent solubility. Collagen uptake was assayed by internalization of fluorescent-collagen-I coated or carboxylated microspheres.

**Results:** hTM cells release vesicles with size (mode = 75nm) and protein composition (CD9, CD81, CD63, AnxA2) characteristic of exosomes. These exosomes bind Fn in a calcium-independent manner. LC-MS/MS revealed a known Fn-binding protein DPPIV, whose activity was detected on purified exosomes. Bound Fn exists in several conformations, including a deoxycholate and SDS insoluble polymer, indicating the presence of both the plasma and cellular forms of Fn. Interestingly, a concurrent increase in hydrodynamic radius was measured in exosomes bound to Fn. Finally, while addition of excess hTM-derived exosomes to hTM cells had no effect on Fn and collagen-IV secretion, excess hTM-derived exosomes increased uptake of collagen-I coated but not carboxylated microspheres by hTM cells.

**Conclusions:** Data support the hypothesis that hTM cells release exosomes at sites of focal ECM degradation to coordinate the uptake of digested ECM components. We speculate that dysfunction in this system is involved in the aberrant accumulation of ECM in the conventional outflow tract and ocular hypertension seen in many forms of glaucoma.

**Commercial Relationships:** W M. Dismuke, None; W D. Stamer, None

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Baicalein lowers intraocular pressure and increases outflow facility in mouse eye

**Hoi Lam Li**, Nicole E. Ashpole, Iris D. Navarro, Thomas C Lam, Ho Lung Henry Chan, Chi Ho To, W D. Stamper, Chi-wai Do

School of Optometry, The Hong Kong Polytechnic University, Hong Kong, Hong Kong; Department of Ophthalmology, Duke University School of Medicine, Durham, NC

**Purpose:** Baicalein is a natural flavonoid derived from the root of Scutellaria baicalensis. We have previously demonstrated that intraperitoneal administration of baicalein lowers intraocular pressure (IOP) in rodents. In this study, we aimed to investigate whether 1) similar ocular hypotensive effects were observed after topical application rather than intraperitoneal injection; and 2) baicalein altered the outflow facility using freshly enucleated mouse eyes.

**Methods:** Adult C57BL/6J (B6) mice were used. Intraocular pressure (IOP) was measured by rebound tonometry under awake condition. Topical baicalein (20 μL, 10 mM) was applied to the treatment eye twice separated by 10-min intervals while phosphate buffered saline (PBS) was used in the fellow eye as control. IOP measurements were conducted before and after drug administration (i.e. 1.5, 3, 6, 24, 48 and 72 h) in both light and dark phases. The outflow facility was determined by measuring the flow rates at sequential pressure steps (i.e. 4, 8, 12, 16 and 20 mmHg) in freshly enucleated mice eyes. Comparisons were made between the baicalein-treated and vehicle-treated contralateral eye of the same animal.

**Results:** Topical administration of 10 mM baicalein caused significant IOP reduction within 6 hours after drug treatment. The maximum IOP-lowering effect was 1.44±0.25 (n = 23, p < 0.01) and 2.16±0.37 (n = 23, p < 0.01) mmHg under light and dark conditions, respectively. In addition, 10μM baicalein significantly enhanced the outflow facility in paired mouse eyes (n = 4, p < 0.05). The outflow facility of baicalein-treated and control eyes were 0.041±0.010 and 0.021±0.005 μL/min/mmHg, respectively.

**Conclusions:** Topical application of baicalein triggered a transient IOP reduction although its effect was smaller than that of intraperitoneal injection. The ocular hypotensive effect was mediated, at least in part, by the facilitation of aqueous outflow. The precise mechanism of action remains to be determined.

**Commercial Relationships:** Hoi Lam Li, None; Nicole E. Ashpole, None; Iris D. Navarro, None; Thomas C Lam, None; Ho Lung Henry Chan, None; Chi Ho To, None; W D. Stamper, None; Chi-wai Do, None

Support: General Research Funds (B-Q34C and B-Q392), PolyU Internal Grants (G-YBGT, G-YK88) and Research to Prevent Blindness Foundation.

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**Trial:** We hypothesized that breathing NO lowers IOP in an sGC-dependent manner.

**Methods:** Anesthetized IOP model: 10- to 20-week-old male wild type (WT) mice and mice deficient in the α1 subunit of sGC (sGCα1-/-; n=9, each) were anesthetized with isoflurane using a standard protocol resulting in a stable IOP baseline. Ten minutes after baseline measurement, IOP was measured again in mice breathing 1.8 % isoflurane and either control gas (N2, balanced in O2) or 40 ppm NO balanced in O2. Awake IOP model: WT mice were acclimated to awake IOP measurements (every other day for 2 weeks). 40 min after baseline measurements, IOP was measured in mice breathing either control gas or 40 ppm iNO (n=8, each) in an incubation chamber.

**Results:** Breathing control gas did not affect IOP in WT or sGCα1-/- mice (Figure 1). Breathing iNO decreased IOP in both anesthetized WT mice (9.86±0.51 vs 8.42±0.51 mmHg at baseline and after iNO, respectively, Figure 1A) and awake WT mice (14.13±0.95 vs 10.93±0.01 mmHg, at baseline and after 40 min iNO, respectively, Figure 1B). In contrast, iNO did not lower IOP in sGCα1-/- mice (9.75±0.31 vs 9.46±0.30 mmHg at baseline and after iNO, respectively, Figure 1A).

**Conclusions:** Inhalation of 40 ppm iNO decreased IOP in anesthetized and awake WT mice but not in sGCα1-/- mice. These findings confirm that NO is an IOP-lowering agent and identify NO-gas as a possible therapeutic approach to acutely lower IOP. In addition, our results identify sGC as the downstream target of NO’s ability to lower IOP. sGC stimulators, under development for treatment of cardiovascular diseases, such as the recently approved ADEMPAS®, may be considered as a novel treatment option for elevated IOP.

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**Commercial Relationships:** Wolfgang-Sebastian Lieb, None; Stefan Munster, None; Anna Dordea, None; Sara Vandenwijngaert, Robert Tainsh, Warren M. Zapol, Emmanuel S. Buys, None

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Methods: Stably-transduced RFP-GRalpha, human and bovine primary TM cell-lines were developed. Levels of importins and exportins in cells treated overnight with vehicle (ethanol) or DEX were studied using western blot in isolated nuclear and cytosolic fractions.

Results: Bovine TM cell-lines have identical GRalpha and beta receptors to human TM cell-lines. DEX increased protein levels of importin b1 (involved in Smad import and ERAD retro-translocation for protein degradation), importin-3 (involved in mRNA transport), and Importin-7 (involved in Smad nuclear import). Also, DEX increased exportin-4 (involved in Smad export), Exportin-5 (involved in pre-miRNA export for maturation in the cytosol), and Exportin-6 (involved in export of actin).

Conclusions: Mechanism of steroid-regulated gene expression is partially mediated through the importin/exportin network in the trabecular meshwork.

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

Support: NIH EY016242

Program Number: 4856 Poster Board Number: A0009
presentation Time: 3:45 PM–5:30 PM

Investigation of the Schlemm’s canal endothelial cells functions using a three-dimensional microfluidic model

Chen-Yuan C. Yang 1, Janet Jeong 2, Roger D. Kamm 2, 4, Haiyan Gong 2, 4 1Anatomy and Neurobiology, Boston Univ School of Med, Boston, MA; 2Ophthalmology, Boston University School of Medicine, Boston, MA; 4Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA; 4Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA.

Purpose: To develop a microfluidic-based assay to study Schlemm’s canal endothelial barrier functions and giant vacuole (GV) formation under physiologically relevant basal-to-apical transendothelial flow and their responses to drug treatment.

Methods: Human Schlemm’s canal endothelial cells (HSCEC) and human dermal lymphatic microvascular endothelial cells (HLMEC) were cultured on collagen type I gel scaffold (2.5mg/mL) in microfluidic devices (based on previous design from Vickerman 2008 with modifications 1). Drug-treated group was incubated with Y-27632 (25uM) for one hour prior testing for diffusive permeability (70kDa dextran) and hydraulic conductivity (9.5mmH2O basal-to-Y-27632 (25uM) for one hour prior testing for diffusive permeability (2.64x10^-5 ± 4.68x10^-6 cm/s, n=4) and hydraulic conductivity compared to the controls. GVs exhibited the classic “signet ring” appearance; interestingly, GV numbers did not seem to differ between Y-27632 treated and control group for both HSCEC (6.5 vs. 7.5 per mm) and HLMEC (21.5 vs. 20.8 per mm).

Conclusions: We have developed a physiologically relevant in vitro three-dimensional microfluidic model to investigate Schlemm’s canal endothelial barrier functions, GV formation, and their responses to drug treatment. This system creates a “trabecular outflow pathway-on-a-chip” and offers innovative ways to study cellular physiology and biomechanics of normal and glaucomatous HSCEC in the future.


Commercial Relationships: Chen-Yuan C. Yang, None; Janet Jeong, None; Roger D. Kamm, AIM Biotech (I); Haiyan Gong, None

Support: NIH EY022634, The Massachusetts Lions Eye Research Fund

Program Number: 4857 Poster Board Number: A0010
presentation Time: 3:45 PM–5:30 PM

The Compensatory Role of Endothelium-Derived Hyperpolarizing Factor (EDHF) in Cholinergic Vasodilatation of the Mouse Ophthalmic Artery

Caroline Manicam, Evgeny Goloborodko, Norbert Pfeiffer, Adrian Gericke. Experimental Ophthalmology, University Medical Center Mainz, Mainz, Germany.

Purpose: The modulation of ocular blood flow is largely attributed to the vascular endothelium. In the mouse ophthalmic artery, endothelial nitric oxide synthase (eNOS) mediates only a part of the cholinergic dilatory response, while another yet unknown mechanism largely contributes towards acetylcholine-induced vasodilation. The aim of this study was to identify the compensatory mechanisms of endothelium-dependent vasodilation in mouse ophthalmic artery when eNOS is lacking.

Methods: Cholinergic vasodilatory responses of ophthalmic arteries from eNOS knockout mice (eNOS-/-) and respective wild type (WT) controls were studied in vitro using isolated vessels. Vascular preparations were incubated with various pharmacological inhibitors, and changes in luminal artery diameter in response to the endothelium-dependent vasodilator acetylcholine (ACh) were measured using video microscopy.

Results: The ACh-induced vasodilation was completely abolished in endothelium-denuded vessels whereas the nitric oxide donor, sodium nitroprusside, produced marked dilation in vessels with and without intact endothelium in WT and eNOS-/- mice. However, inhibition of NOS only caused a partial attenuation of the vasodilator responses in the WT mice but had no effect in the eNOS-/- mice. The involvement of EDHF was confirmed with 30 mM of potassium solution that completely abolished vasodilation in both mouse genotypes in the presence of NOS and cyclooxygenase (COX) inhibitors. Our data suggest that the arachidonic acid metabolites generated via the cytochrome P450 (CYP450) and lipoxygenase (LOX) pathways, the voltage-gated potassium channel Kv1.3 and gap junctions are the key players in modulating the ophthalmic artery diameter in the WT mice. In contrast, the lack of eNOS is compensated by the LOX pathway and voltage-gated potassium channels, but without CYP450 and gap junction involvement.

Conclusions: This study provides first evidence that the compensatory EDHF mechanisms differ between WT and eNOS-/- mice. While ACh-induced vasodilation in WT mouse ophthalmic arteries is mediated in part by NO and predominantly by EDHF, only EDHF mediates the response in eNOS-/- mice.

Commercial Relationships: Caroline Manicam, None; Evgeny Goloborodko, None; Norbert Pfeiffer, None; Adrian Gericke, None

Support: None, None

Commercial Relationships: None; None

Presentation Time: 3:45 PM–5:30 PM

Poster Board Number: A0009

Commercial Relationships: None; None

Presentation Time: 3:45 PM–5:30 PM

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OPTIC NERVE HEAD AND CHOROIDAL VASOREACTIVITY TO HYPOXIA IN HEALTHY HUMANS

Mathilde Gallice1, Thierry Zhou1, Florent Aptel1, Samuel Verges1, Martial Henry Geiser2, Jean Paul Romanet3, Christophe Chiquet4, 5
1GRENoble, University Hospital, Grenoble, France; 2Haute Ecole Valaisanne, Sion, Switzerland; 3INSERM U1042, Grenoble, France.

Purpose: Optic nerve head (ONH) and choroidal (Ch) vasoreactivity to hypoxia is not clearly defined, although it is a common pathological situation in diabetic retinopathy, retinopathy of prematurity, open-angle glaucoma or in systemic diseases. We measured ocular blood flow changes in response to acute hypoxia in healthy humans.

Methods: Using confocal laser Doppler flowmetry, ONH and Ch blood flow were measured under hypoxia condition during 15 minutes (FiO2 10.5%) in order to reach a stable 85% SpO2, PetCO2, blood pressure, and respiratory rate were monitored during the experiment.

Results: Fourteen young (26±6 years) healthy subjects were included. In response to 85% hypoxia, ONH blood flow increased of +11% [-2.4; 34] (median and interquartiles interval, p=0.08) mainly due to an increase in ONH velocity +18% [6; 27] (p=0.004). Choroidal LDF parameters did not significantly differ during hypoxia (p=0.2 for Ch flow, p=0.3 for Ch volume, and p=0.12 for Ch velocity). Respiratory rate or arterial pressure did not significantly change during experiment.

Conclusions: The response of ONH to hypoxia is close to that described for the retina. The absence of choriocapillaris vasoreactivity to hypoxia, despite a significant decrease in tissue oxygen partial pressure, could be explained by the counterbalanced vasodilation associated with the activation of the autonomic nervous system during hypoxia.

Commercial Relationships: Mathilde Gallice, None; Thierry Zhou, None; Florent Aptel, None; Samuel Verges, None; Martial Henry Geiser, None; Jean Paul Romanet, None; Christophe Chiquet, None

The reactivity of retinal arterial and venous diameter, red blood cell (RBC) velocity and flow to 100% O2 breathing was investigated in the absence and presence of 2ng/kg LPS. Study participants were randomized to receive either placebo or nutritional supplements for 14 days. After that period, measurements were repeated.

Results: The decrease in retinal arterial diameter, RBC velocity and flow induced by breathing 100% O2 was significantly blunted after LPS infusion. Intake of the dietary supplement for 14 days almost restored the response of retinal hemodynamic parameters to 100% O2 after LPS administration. This effect was significant for retinal arterial diameter (p = 0.03 between groups), RBC velocity and flow (each p < 0.01 between groups).

Conclusions: In conclusion, the present study shows that 14 days intake of nutritional supplements reverses LPS induced alterations in the reactivity of the retinal vasculature. The obtained results indicate that the used combination of antioxidants is capable of restoring retinal vascular endothelial function during oxidative stress.

Commercial Relationships: Doreen Schmidl, None; Reinhard Told, None; Stefan Palkovits, None; Agnes Boltz, None; Rene Werkmeister, None; Gerhard Garhofer, None; Leopold Schmetterer, None

Support: An unrestricted research grant from Ursapharm Arzneimittel GmbH is gratefully acknowledged

Clinical Trial: NCT02221089

The Effect of Antioxidant Supplementation on Oxygen Induced Retinal Vasocostriction in an Inflammatory Model in Humans

Doreen Schmidl1, 2, Reinhard Told1, 2, Stefan Palkovits1, 2, Agnes Boltz1, 2, Rene Werkmeister1, Gerhard Garhofer1, Leopold Schmetterer1, 2
1Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria; 2Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria; 3Department of Ophthalmology, Medical University of Graz, Graz, Austria.

Purpose: Dietary supplements have been found to be beneficial in age-related macular degeneration (AMD) due to their anti-oxidative properties. In the AREDS 1 study, a reduced progression to late stage AMD was associated with the use of a dietary supplement containing vitamin C, E, zinc and β-carotene. In a previous study, we have shown that this AREDS 1 formulation restores the O2-induced retinal vasocostructor response of retinal vessels in a human endotoxin (lipopolysaccharide, LPS) model. The aim of the present study was to test the hypothesis that this abnormal O2-induced retinal vasocostructor response to LPS can also be modulated by a different formulation containing vitamin C, E, zinc, lutein/zeaxanthin, selenium, taurine, Aronia extract and omega-3 free fatty acids.

Methods: In the present randomized, double masked, placebo-controlled parallel group study, 43 healthy subjects were included.
study intracranial hypertension or glaucoma by quantification of the global arterio-venous flow of the eye.

A coronal plane of acquisition has been placed perpendicularly to the direction of the ophthalmic artery (OA) or the superior ophthalmic vein (SOV), in order to acquire phase contrast magnetic resonance imaging (PC MRI) images. The region of interest is selected and segmented. The segmentation is applied on the whole sequence i.e. 32 images corresponding to 32 phases of an average cardiac cycle. The blood flow is calculated and the software provides the curve of the evolution of the flow during cardiac cycle.

Evolution of flow (in mL/min) during cardiac cycle in right and left superior ophthalmic vein of one healthy subject.

Commercial Relationships: Veronique Promelle, None; Solange Milazzo, None; Gwenael Page, None; Joel Daouk, None; Roger Bouzerar, None; Olivier Balédent, None

Program Number: 4861 Poster Board Number: A0014
Presentation Time: 3:45 PM–5:30 PM

Novel measurement technique of retinal vessel caliber using fundus photographs obtained from optical coherence tomography
Salman Sarwar1, Mostafa S. Hanout1, Mohamed K. Soliman1, Mohammad A. Sadiq1, Robin High1, Aniruddha Agarwal2, Diana V. Do2, Quan Dong Nguyen2, Yasir J. Sepah2. 1Department of Biostatistics, College of Public Health, University of Nebraska Medical Center, Omaha, NE; 2Department of Ophthalmology, Stanley M. Truhlsen Eye Institute, University of Nebraska Medical Center, Omaha, NE.

Purpose: Measurement of retinal vessel caliber has been previously performed on color fundus photographs using the Interactive Vessel Analysis (IVAN) software. In this cross-sectional study, we compared and assessed the correlation between manual measurements on confocal scanning laser ophthalmoscopy (cSLO) fundus images and the IVAN technique.

Methods: A total of 36 eyes (36 subjects) were included in the study: 11 subjects with no known ocular or systemic diseases, 13 with non-vascular age-related macular degeneration (AMD), and 12 with systemic hypertension without any known ocular diseases. 30 optic disc-centered fundus photos (Field 1M) were obtained by cSLO (Heidelberg Spectralis®) using multicolor imaging mode. Color fundus photographs of the same eyes were also acquired using Carl Zeiss FF450 fundus camera. IVAN was used to measure retinal vessel caliber from the color fundus photos, whereas measurement tool of the Heidelberg Eye Explorer (HEYEX v5.2) was independently used to measure manually the retinal vessel calibers from the cSLO images (Figure). All measurements were taken in an area of 0.5 to 1 disc diameter from the margin of optic disc for both color photos and cSLO images. Central retinal arteriolar equivalents (CRAE) and central retinal venular equivalents (CRVE) were calculated using a standardized formula for both image modalities. Correlation between the CRAE and CRVE values obtained by the two methods was analyzed using the Pearson correlation coefficient.

Results: The mean age was 32, 59, and 73 years for the normal, hypertensive and non-vascular AMD groups, respectively. The mean difference for CRAE between IVAN and cSLO method ranged from 19.1 to 22.7 μm. Mean differences for CRVE between the two methods ranged from 19.6 to 21.6 μm. There was a strong correlation between IVAN and cSLO for CRAE and CRVE measurement across the three groups (Table).

Conclusions: Measurement of retinal vessel caliber using Heyex measurement tool on cSLO fundus images may provide an alternative method to the standard IVAN software. The Heyex technique requires less image processing and is less time consuming. The cSLO images can also be obtained without the need for mydriasis.

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Purpose: The etiology and mechanism of hypertensive retinopathy are not fully understood. Transverse aortic coarctation (TAC) between the left common carotid artery and the right carotid artery. Sham-operated mice served as controls. Central retinal artery (CRA) blood velocity was measured by Doppler ultrasound. Retinal fundus imaging, spectral domain optical coherence tomography (SD-OCT), and histological examination were used to assess retinal abnormalities.

Methods: Mice were subjected to TAC for 1 or 3 months by banding the aortic arch between the left common carotid artery and the right brachiocephalic artery, which gives rise to the right common carotid artery. Sham-operated mice served as controls. Central retinal artery (CRA) blood velocity was measured by Doppler ultrasound. Retinal fundus imaging, spectral domain optical coherence tomography (SD-OCT), and histological examination were used to assess retinal abnormalities.

Results: Induction of TAC in mice for 1 month had no effect on the body weight but caused cardiac hypertrophy with increased heart-to-body weight ratio by 45% and decreased left ventricular fractional shortening by 51%. As compared to the sham-operated mice, blood flow velocity in the right CRA of TAC mice was increased by 45% at 1 month and 3 months after surgery. For the left eye, CRA blood velocity had no change at 1 month but was decreased by 23% at 3 months after aortic banding. Fundus imaging showed no apparent retinal abnormality between sham and TAC mice. Attenuation of the retinal nerve fiber layer and the inner retina was found in the right eye of TAC mice by SD-OCT. Histological examination of the retina indicated a decrease in the thickness of the inner nuclear layer in both right and left eyes in TAC mice at 3 months after surgery. Cell loss and morphological changes were found in the ganglion cell layer (GCL), indicating GCL degeneration in the TAC mice.

Conclusions: Elevated right carotid arterial blood pressure by TAC leads to increased CRA blood flow and retinal degeneration in mice. In the later phase, the compensatory reduction of left carotid arterial perfusion, which leads to left CRA blood flow deficiency, also causes retinal injury. This experimental model may be useful for studying the mechanisms and potential treatments of retinopathy in association with chronic changes in systemic blood pressure and ocular perfusion.

Commercial Relationships: Shu-Huai Tsai, None; Wankun Xie, None; Robert H. Rosa, None; Travis W. Hein, None; Lih Kuo, None
Support: Supported by Retina Research Foundation and Kruse Centennial Chair Fund

Program Number: 4863 Poster Board Number: A0016
Presentation Time: 3:45 PM–5:30 PM
Differences between arteries and veins of retinal blood flow determined by laser speckle flowgraphy in healthy eyes Takeshi Iwase, Eimei Ra, Kentaro Yamamoto, Hiroko Terasaki. Ophthalmology, Nagoya University Hospital, Nagoya, Japan.
Purpose: To characterize the total retinal blood flow determined by laser speckle flowgraphy (LSFG) (LSFG Analyzer, Softcare) of healthy subjects.
Methods: Twenty-seven right eyes of 27 healthy subjects (mean age: 30.3 ± 7.6 years) were studied. The total blood flow in the retinal arteries and veins separately around the optic nerve head was measured using the total retinal flow index (TRFI), which represents blood flow volume, and comparisons were made between the TRFI of the arteries and veins. The lumen diameters of the retinal vessels determined by LSFG and by adaptive optics (AO) camera (rtx1, Imagine Eyes) were compared. The images obtained by LSFG and AO camera were merged, and the distribution of the mean blur rates (MBRs), which represent the velocities of the erythrocytes, was evaluated on the images.
Results: The mean TRFI in veins (1880±411, arbitral units) was significantly higher than that in arteries (1581±346, arbitral units; P<0.001). Linear regression analysis showed a significant correlation between the TRFI in the arteries and veins (P<0.001). Linear regression analysis also showed a highly significant correlation between the diameters of arteries and veins determined by LSFG and by the AO camera (arteries, r=0.91, P<0.001; veins, r=0.87, P<0.001). The ratios of the lumen diameters determined by LSFG to that by AO camera was significant lower in arteries (0.068±0.006, arbitral units) than in veins (0.076±0.011, arbitral units) (P=0.011). The MBRs of veins were homogeneous throughout the width of the lumen, however the MBRs in the arteries were higher at the center and lower close to the walls of the lumen.
Conclusions: The higher TRFIs in the veins than in the arteries indicate that there is a smaller volume of retinal blood flow in arteries than veins. However, the possibility remains that LSFG has inherent problem that the arterial lumen diameter determined by LSFG is smaller than actual one because of the characteristics of arteries. This would result in a smaller volume of retinal blood flow in the arteries than veins in LSFG.

Commercial Relationships: Takeshi Iwase, None; Eimei Ra, None; Kentaro Yamamoto, None; Hiroko Terasaki, None

Program Number: 4864 Poster Board Number: A0017
Presentation Time: 3:45 PM–5:30 PM
Purpose: Although cigarette smoking has been identified as a major risk factor for atherosclerotic complications in coronary, aortic and cerebral circulation, the relationship of cigarette smoking and ocular circulation remain to elucidate in detail. The current study examined the effect of chronic smoking on the retinal microcirculation in patients with type 2 diabetes with early stages of retinopathy.
Methods: Using a laser Doppler velocimetry system, we obtained the retinal blood flow (RBF) values by simultaneously measuring the retinal blood flow (RBF) values by simultaneously measuring
We compared the safety and tolerability of low dose brimonidine tartrate ophthalmic solution 0.025%, with its vehicle in a population of pediatric, adult, and geriatric subjects.

**Methods:** The study was a Phase 3 multi-center, double-masked, randomized, vehicle-controlled, parallel-group study in pediatric (5–17 yrs), adult (≥18–≤65 yrs), and geriatric (≥65 yrs) healthy subjects, that comprised 4 study visits over ~4 weeks. Subjects were randomized 2:1 (active:vehicle) to receive one drop of brimonidine tartrate 0.025% or vehicle, bilaterally, 4 times daily, for up to 4 weeks. Drop comfort (0-10 scale) was assessed at Visit 1 immediately following instillation and 30 seconds and 1 minute post-instillation. At all study visits, safety assessments, adverse events (AEs) query, alertness evaluation, IOP, and vital signs measurements were performed by the investigator. Blood and urine samples were collected from a subset of subjects for clinical hematology, blood chemistry, and urinalysis. Subjects used a dosing diary to record in-home dosing between study visits.

**Results:** A total of 507 subjects were randomized, including 50 pediatric and 49 geriatric subjects. Two subjects in the brimonidine group reported 3 treatment-emergent serious adverse events (SAEs); all were non-ocular and considered unrelated to study medication. No ocular treatment emergent AEs were reported in subjects <18 years of age. No safety concerns were raised based on clinical examinations. Investigator evaluations of alertness showed no evidence of brimonidine causing somnolence. In the brimonidine-group, no substantial changes in IOP were reported during the study. Brimonidine was very comfortable (mean score 0.4), and no significant differences in drop comfort were reported between the brimonidine and vehicle groups.

**Conclusions:** Brimonidine tartrate 0.025% ophthalmic solution was safe, comfortable, and well tolerated when dosed 4 times a day for 4 weeks in this study.

**Commercial Relationships:** Quintus Ngumah, Bausch + Lomb (E); Baldo Scassellati-Sforzolini, Bausch + Lomb (E); Paul J. Gomes, Ora, Inc. (E)

**Clinical Trial:** NCT01959243
the proportion of eyes with a ≥1-grade reduction (improvement) from baseline in individual or composite blepharitis signs (Table 1). For patients in the highest severity quartile at baseline and evaluated on Day 15 (n=120), blepharitis signs were fully resolved in 23.7% (14/59) of eyes treated with LE/T and 21.3% (13/61) of the eyes treated with DM/T (P=0.8284). Mean IOP increased significantly with DM/T compared with LE/T beginning at Day 7 (US study) or Day 3 (China study) (P=0.0339). IOP increases of ≥10 mm Hg over baseline were noted in 1 US patient (DM/T group) and 19 Chinese patients (6 LE/T; 13 DM/T).

**Conclusions:** In this pooled analysis, LE/T was effective in reducing the signs of blepharitis with similar results compared to DM/T. LE/T had a better safety profile with respect to change in IOP especially in Chinese patients considered at higher risk for steroid-induced IOP.

**Table 1. Percent of Study Eyes with a ≥1-Grade Reduction (Improvement) from Baseline to Day 15 in Blepharitis Signs**

<table>
<thead>
<tr>
<th></th>
<th>Percent, % (n/N)</th>
<th>Two-Tailed P-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LE/T</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lid hyperemia</td>
<td>82.0 (191/233)</td>
<td>79.2 (183/231)</td>
</tr>
<tr>
<td>Lid scaling/crusting</td>
<td>63.9 (149/233)</td>
<td>68.4 (158/231)</td>
</tr>
<tr>
<td>Lid margin hypertrophy</td>
<td>43.3 (101/233)</td>
<td>45.9 (106/231)</td>
</tr>
<tr>
<td>Composite score</td>
<td>93.6 (218/233)</td>
<td>95.2 (220/231)</td>
</tr>
<tr>
<td><strong>DM/T</strong></td>
<td></td>
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* Fisher’s Exact Test

**Commercial Relationships:** Timothy Comstock, Bausch + Lomb (C); Helen H. DeCory, Bausch + Lomb (E)

**Clinical Trial:** NCT01028027, NCT00447577

**Program Number:** 4868 Poster Board Number: A0021

**Presentation Time:** 3:45 PM–5:30 PM

**Clinical and experimental evidence for post-stroke demyelination in nonarteritic anterior ischemic optic neuropathy (NAION)**

Gregory L. Fu, Steven L. Bernstein. Department of Ophthalmology and Visual Sciences, University of Maryland School of Medicine, Baltimore, MD.

**Purpose:** Many NAION patients experience a progressive decline in vision during the first three months post-infarct, but the mechanism for this decline is poorly understood. Using visual evoked potentials (VEPs), our lab recently determined that isolated optic nerves (ONs) from the rat NAION (rAION) model exhibit both reduced VEP amplitudes and slowing of conduction velocity. We wanted to test the hypothesis that post-infarct ON demyelination could be responsible for progressive post-infarct vision loss with preservation of axons using animal models and a human donor diagnosed with NAION.

**Methods:** We analyzed ON tissue sections from rAION models, primate NAION (pAION) models, and a human donor previously diagnosed with bilateral NAION. Controls included tissue from uninjured contralateral eyes, and tissue from human donors without history of eye disease. We evaluated demyelination using both ON ultrastructure analysis via transmission electron microscopy (TEM), and immunohistochemistry for axons (SMI312) and myelin basic protein (MBP). We evaluated myelin thicknesses, and demyelination ratios were measured from TEM images using measuring wheel and architectural scale (Scalpel MapWheel at a scale of 1:1500).

**Results:** TEM analysis and quantification revealed marked signs of axonal loss and demyelination post-stroke in both rAION and pAION models. At both 1650x and 6500x magnifications, there was noticeable myelin loss and unwinding in individual ON axons in the presence of normal and healthy axons. Demyelination ratios were significantly different (p<0.0001) between the pAION model and controls, regardless of axon size (n=42 per size): small, 72.8±4.0%; medium, 73.8±3.9%; large, 78.7±3.8%. Immunohistochemistry analysis of pAION ON slices demonstrated discernible areas of both healthy myelinated axons and demyelinated functional axons.

**Conclusions:** NAION and its models induce post-infarct demyelination with regional axonal preservation seen in the NAION animal models. Our results are consistent with previously determined VEP results, suggesting that ON axonal demyelination likely contributes to visual loss. Useful future treatments for NAION may focus on preventing or reducing the demyelination component of ON damage, and provide an alternative novel treatment option for improving progressive vision loss in NAION patients after the initial ischemic event.

**Commercial Relationships:** Gregory L. Fu, None; Steven L. Bernstein, None

**Support:** This study was supported in part by the NIA Short-Term Training Program on Aging Grant T35AG036679 (J.E. Warnick, P.I.) to the University of Maryland School of Medicine.

**Program Number:** 4869 Poster Board Number: A0022

**Presentation Time:** 3:45 PM–5:30 PM

**Retrospective review of interventional thrombolysis for central retinal artery occlusion at the University of Kansas Medical Center**

Lillian Yang. Ophthalmology, University of Kansas, Prairie Village, KS.

**Purpose:** Thrombolysis has been proposed as a treatment in patients with central retinal artery occlusion (CRAO). The aim of this study is to review the University of Kansas’ experience in treating CRAO using intra-arterial tPA to assess the safety and effectiveness of the procedure by measuring outcomes and complications.

**Methods:** Records from seven patients diagnosed with central retinal artery occlusion and treated with intra-arterial tPA were retrospectively reviewed for ophthalmic findings before and after treatment as well as complications after treatment.

**Results:** Two patients had stable visual acuity before and after treatment given 9 and 5 hours, respectively, after onset of symptoms. Three patients had improved vision from hand motion to 20/20, count fingers at 1 ft to count fingers at 4 ft, and light perception to hand motion, given 11 hours, 14 hours, and unknown duration respectively after onset of symptoms. The patient with hand motion improvement to 20/20 was noted to have a patent cilioretinal artery on funduscope exam. Two patients had a decrease in vision, both light perception to no light perception, treated 7.5 hours and 8 hours, respectively, after onset of symptoms. The range of time to tPA after onset of symptoms was 1.5 hours to 17 hours. All patients were worked up with ESR and CRP to rule out giant cell arteritis. One patient experienced oral cavity hemorrhage day one after intra-arterial tPA but did not have intracranial hemorrhage.

**Conclusions:** Overall, most patients that received treatment with intra-arterial tPA did not have significant improvement in visual acuity when checked 1-4 days post treatment. One patient did experience a significant improvement in visual acuity however, the patient had a patent cilioretinal artery on funduscope exam which may have been responsible for the improvement. One patient suffered a minor complication of oral hemorrhage without intracranial hemorrhage.

**Commercial Relationships:** Lillian Yang, None
Program Number: 4870 Poster Board Number: A0023
Presentation Time: 3:45 PM–5:30 PM
Management of Congenital Nasolacrimal Duct Obstruction with Infection in Infants using Besifloxacin-A Prospective Study
Yufei Tu1, Boschert Boschert1, Joseph Schwab2, Rudolph Wagner1, Patrick DeRespinis3, suqin guo1. 1Institute of Ophthalmology & Visual Sci, Rutgers University, New Jersey Medical School, Newark, NJ; 2Pediatrics, Rutgers University, New Jersey Medical School, Newark, NJ; 3Pediatric Ophthalmology, Staten Island, Staten Island, NY.

Purpose: There is no standard of choice of antibiotics for the treatment of congenital nasolacrimal duct obstruction (NLDO) with infection among children under-1-year. This study analyzes the efficacy and safety of a fourth-generation fluoroquinolone, Besifloxacin (Besivance) and compares Besivance with Trimethoprim /polymyxin (Polytrim) in the treatment of NLDO with infection.

Methods: Twenty-four children under 1-year old with a diagnosis of congenital NLDO with infection were randomized to receive either Besivance or Polytrim (3 times a day for 10 days). A NLDO severity scale based on a standard set of photos, with grades ranging from 0 (no infection) to +4 (severe infection) was developed prior to subject enrollment. NLDO severity scores were evaluated for each subject by treating physician at baseline, 2-, 8- and 16-week visits. The primary outcome measure was a change in NLDO grading score from baseline to 8-week visit. Treatment success was defined as a score of zero after baseline visit or score improvement of 2 or more from the prior visit.

Results: The average age at baseline was 4.5 months. Four subjects were excluded from data analysis (three were lost to follow-up and one was withdrawn by PI). At baseline, the mean NLDO score of Polytrim group (3.0, SD=0.89) was higher than Besivance group (2.44, SD=0.88), indicating worse NLDO in Polytrim group. To adjust for this, the weighted mean change in NLDO scores from baseline to 8-week visit was compared. There was no statistically significant difference in the mean weighted NLDO score (P=0.27) by group (Besivance weighted mean=0.81 (SD=0.39) vs. Polytrim weighted mean= 0.95 (SD=0.12)). Eight (8/9, 88%) Besivance subjects, and ten (10/11, 91%) Polytrim subjects were treated successfully. One (1/9, 11%) Besivance subject, and one (1/11, 9%) Polytrim subject suffered recurrence. No serious adverse events were reported in either group.

Conclusions: Compared to Polytrim, Besivance is as effective and safe in treating NLDO with infection in children less than 1 year of age. Because Besivance is formulated in the DuraSite vehicle allowing higher concentration and prolonged contact time on the eye, less frequent dosing is needed hence better compliance in this young patient population.

 Commercial Relationships: Yufei Tu, None; Boschert Boschert, None; Joseph Schwab, None; Rudolph Wagner, None; Patrick DeRespinis, None; suqin guo, Bausch & Lomb Incorporated (F)
Support: Bausch & Lomb Incorporated
Clinical Trial: NCT01431170