

AOPT | Association for Ocular Pharmacology and Therapeutics

12th Scientific Meeting

February 26th - March 1st, 2015

Charleston Marriott
Charleston, SC



NC STATE UNIVERSITY



Association for Ocular
Pharmacology and Therapeutics

www.aopt.org

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Welcome Message



Welcome to Charleston, SC and the 12th Scientific AOPT Meeting. This year marks the 20th anniversary of the first official meeting of the then newly founded Association for Ocular Pharmacology and Therapeutics, AOPT, which was held in New Orleans, LA in January of 1995. The origins of AOPT date back to an inaugurating Ocular Pharmacology Symposium organized by founding members Hitoshi Shichi and George Chiou in Novi, Michigan in 1993. The goal was, and remains to this day, to bring together scientists (basic, preclinical, clinical) from academia and the pharmaceutical industry and eye care professionals who share an interest in ocular pharmacology and the treatment of ophthalmic diseases and to promote an exchange of scientific information. The biennial AOPT meetings provide an international forum for close interaction with colleagues and friends who share a passion for finding new treatment solutions for patients with vision related problems and diseases. These personal interactions strengthen collaborations and friendships or lead to new ones.

The program spans two and a half days starting with a keynote presentation by Krzysztof Palczewski from Case Western Reserve University, the 2014 Friedenwald award recipient. New this year, both lunches on Friday and Saturday will feature special informational sessions, one on fostering collaborations between academia and industry, the other a Lunch-n-Learn session featuring a mix of topics. Thanks to our sponsors, I am particularly excited to announce that 30 travel grants were awarded this year to young investigators and junior faculty members. I believe you will find the program stimulating and informative, walk away with new ideas, make new contacts while strengthening existing ones, and forge new relationships and collaborations.

I want to take this opportunity to express my sincere thanks to the organizing committee, chaired by Dan Stamer, for all their efforts and countless hours spent in putting together an exciting meeting at a fabulous venue.

More information on previous meetings and the society can be found on the AOPT website and elsewhere in this program book. If you are not a current member of AOPT I encourage you to sign up or renew your membership. Membership comes with many benefits. To find out more please visit www.aopt.org.

See you in Charleston.

Achim Krauss

AOPT President

AOPT Membership Information

The bylaws of the association establish four classes of membership in AOPT: Regular Members, Associate Members, Contributing Members, and Emeritus Member.

- **REGULAR MEMBERS.** Regular Members are individuals demonstrating a genuine interest in or making significant contribution to ocular pharmacology and therapeutics. This may be evidenced by a) scientific publications; b) attendance at pharmacological, ophthalmological, optometric, or visual science meetings; c) direct involvement in research. A candidate for membership completes the online membership form and pays the appropriate membership dues. Membership is for two years. A subscription to the Journal of Ocular Pharmacology and Therapeutics is optional.
- **ASSOCIATE MEMBERS.** Associate Membership is for predoctoral and postdoctoral students. A candidate for this membership must have a pre-doctoral, or post-doctoral student status, and must complete the online membership form and pay the appropriate membership dues.
- **CONTRIBUTING MEMBERS.** Contributing Membership is restricted to corporations, associations, and individuals who support the objectives of AOPT but do not satisfy the requirements of Regular Membership or individuals elected to membership in any class who voluntarily choose to become Contributing Members. A candidate for contributing membership completes the online membership form and pays the appropriate membership dues.
- **EMERITUS MEMBERS.** Any Regular Member may make a written request to the Treasurer that his/her membership be transferred to that of an Emeritus Member. The request is subject to approval of the membership committee. Emeritus Members have all the rights and privileges of Regular Members, except those of voting and holding elective office.

Membership application form is available at the Association for Ocular Pharmacology and Therapeutics website (<http://www.aopt.org/membership/apply/?Membership-Application>).

Official Journal

The Journal of Ocular Pharmacology and Therapeutics (JOPT), published by Mary Ann Liebert, Inc., publishers (140 Huguenot Street, 3rd Floor, New Rochelle, NY 10801), is the official Journal of AOPT. A substantially reduced subscription rate for this journal (electronic format) is an optional membership benefit, as indicated above.

AOPT Information

Information about the AOPT membership or any other matters related to the Association can be obtained using the on-line contact available at <http://www.aopt.org/contact-us>.

AOPT Past Meetings

Meeting	Date	Location	Organizer
<u>Eleventh Meeting</u>	February 7-10, 2013	Alicante, Spain	Juana Gallar Martinez
<u>Tenth Meeting</u>	February 17-20, 2011	Ft. Worth, TX	Tom Yorio Abbot Clark
<u>Ninth Meeting</u>	February 18-21, 2009	Salzburg, Austria	Herbert Reitsamer
<u>Eighth Meeting</u>	February 9-11, 2007	San Diego, CA	John Liu Achim Krauss
<u>Seventh Meeting</u>	February 3-5, 2005	Catania, Sicily, Italy	Filippo Drago
<u>Sixth Meeting</u>	February 1-4, 2003	Kona, HI	Peter Kador
<u>Fifth Meeting</u>	November 2-5, 2000	Birmingham, AL	Jimmy Bartlett
<u>Fourth Meeting</u>	January 28-31, 1999	Irvine, CA	Achim Krauss
<u>Third Meeting</u>	October 22-24, 1997	Bethesda, MD	Peter Kador
<u>Second Meeting</u>	August 15-17, 1996	Los Angeles, CA	David Lee
<u>First Meeting</u>	January 26-29, 1995	New Orleans, LA	Herb Kaufman
Ocular Pharmacology Symposium	August 8-10, 1993	Novi, MI	Hitoshi Shichi

Your AOPT Board



President
Dr. Achim H. Krauss



Vice-President
Dr. Thomas Yorio



Secretary
Dr. Jeff Kiel



Treasurer
Dr. Ganesh Prasanna



Trustee
Dr. Julie Crider



Trustee
Dr. Filippo Drago



Trustee
Dr. Malinda Fitzgerald



Trustee
Dr. Juana Gallar



Trustee
Dr. Peter Kador



Trustee
Dr. W. Daniel Stamer



Trustee
Dr. Oliver Zeitz

Your Local Organizing Committee



Dan Stamer,
Duke University



Cathy Bowes Rickman,
Duke University



Chris McGahan,
North Carolina State University



Craig Crosson,
Medical University of South Carolina



Shahid Husain,
Medical University of South Carolina

Travel Award Winners

National Eye Institute Awardees

Junior Faculty

Jing Wang, Ph.D., Medical College of Georgia, USA
Morgan Fedorchak, Ph.D., University of Pittsburgh, USA
Jie Fan, Ph.D., Medical University of South Carolina, USA
Dorota Stankowska, Ph.D., University of North Texas Health Sciences Center, USA
Ariel Gore, Ph.D., Israel Institute for Biological Research, Israel
Ghulam Mohammad, Ph.D., King Saud University, Saudia Arabia
Matthew Campbell, Ph.D., Trinity College Dublin, Ireland

Post Doctoral Fellows and Students

Sarah Jacobo, Ph.D., Harvard University, USA
Line Petersen, Aarhus University, Denmark
Doreen Schmidl, M.D., Ph.D., Medical University of Vienna, Austria
Emilie Picard, Ph.D., Centre de Recherche des Cordeliers, France
Stephanie Merrigan, University College Dublin, Ireland
Xavier Gerard, Ph.D., Institut Imagine, France
Hongwei Ma, Ph.D., University of Oklahoma, USA
Jakub Hanus, Ph.D., Tulane University, USA
John Fuller, Ph.D., Johns Hopkins University, USA
Kacie Meyer, Ph.D., University of Iowa, USA

AOPT Awardees

Yuliya Naumchuk, University of Missouri, USA
Teresia Carreon, University of Miami, USA
Karen Torrejon, SUNY, USA
Yong Park, University of North Texas Health Science Center, USA
Manas Biswal, Ph.D., University of Florida, USA
Kai Kang, Ph.D., Cleveland Clinic, USA

BrightFocus Foundation Awardees

Cula Dautriche, SUNY, USA
Michael Dismuke, Ph.D., Duke University, USA
Sumana Chintalapudi, Indiana University, USA
Christopher Toomey, Duke University, USA

MaryAnn Liebert Publishers Awardees

Rania Sulaiman, Indiana University, USA
Nestor Gomez, Ph.D., University of Pennsylvania, USA

The Glaucoma Foundation Awardee

Inas Aboobakar, Duke University, USA

General Information

On-site Registration Desk

Thursday, February 26th, 15:00 – 19:00

Friday, February 27th, 07:30 – 12:30

Late registrants can obtain their badges from on call assistant.

Name Badges

All participants must wear their name badges throughout the meeting. Name badges allow admission to all sessions, breaks, lunches, receptions and the banquet.

Welcome Reception

The Welcome reception will be held on Thursday February 7, 2013 from 5:00p to 7:00p in the Courtyard outside of the Topaz room (in Topaz room if raining)

AOPT Business Meeting

The AOPT Business Meeting will be held on Friday, February 27 from 17:15-17:45 hours. All AOPT members are encouraged to attend. Refreshments will be served.

AOPT Banquet

The AOPT Banquet, open to all registered participants, will be held Saturday, February 28 at 19:00 in the Topaz room. A reception will start at 19:00, preceding dinner, which will be served at 19:30. Travel awards will be presented during coffee and dessert.

Clothing

Clothing is business casual for all occasions.

Internet Access

Free WIFI connection is provided in rooms of all registered participants and in hotel lobby.

Liability and Personal Insurance

The AOPT 2015 Organizers cannot accept liability for personal accidents or loss of or damage to private property of participants and accompanying persons. Participants are recommended to take out their own personal travel and health insurance for their trip to Charleston.

Safety and Security

We kindly request you not to leave bags, suitcases or backpacks unattended at any time during the meeting.

Information for Presenters

Language

The official language of the AOPT 2015 meeting is English.

Oral Presentations

Presenters using a Powerpoint presentation should bring it on memory stick (USB), and load it in the designated computer between 7:30-8:00 am (morning sessions) or during the lunch break (afternoon sessions). Presenters combining Powerpoint and video films are requested to double-check their presentations before the session begins to be sure they work properly. If you are a Macintosh user, please convert your file to Powerpoint to be used on the PC. Alternatively, MAC users can bring their own computer and a VGA adaptor.

Poster Presentations

Posters must be mounted by 11:00 on Friday, February 27 and remain on display until the end of the day (18:30) on Saturday, February 28th. Poster presenters are required to stand by their posters for informal discussions during the designated poster session on Friday, February 27 (17:45-19:30) and requested to check on their poster during the coffee and lunch breaks. Posters left up on Sunday will be removed and discarded. AOPT 2015 is not responsible for poster materials left at meeting's end.

Disclosures

All Commercial Relationships must be indicated on a slide of the presentation and on the posters, even if they were not indicated at abstract submission.

Recording Policy

Recording (photographing, audiotaping, or videotaping) any presentation or poster is PROHIBITED, except by AOPT agents for official purposes or by authors who want to photograph their own poster presentations

Hotel Amenities



Welcome to the Charleston Marriott

Where luxury and southern comfort meet...

Hotel Amenities

- Shuttle Service to Historic District
 - 8:00AM to 10:30PM (See Front Desk for Details)
 - 6 USD All-Day Pass
- Saffire Restaurant and Bar
 - Open for Breakfast from 6:30AM to 11:00AM (12:00PM Saturday and Sunday)
 - All Day Menu from 11:00AM to 10:00PM
- Aqua Terrace Roof-Top Bar
 - Open 4:00PM to 2:00AM (Seasonal/Weather Permitting)
- Daily Grind featuring Starbucks Coffee
 - Open 6:30AM to 11:00PM
- Room Service
 - Open 6:00AM to 11:00PM
- Pool
 - Until 11:00PM with Key Access
- Fitness Center
 - 24 Hours with Key Access
- Outdoor Courtyard with Luxury Seating

Other Amenities

- Check in: 4:00PM/Check Out: 11:00AM
 - Express and Mobile Check In/Check Out Available
- Complimentary On-Site Parking or Valet Parking 10 USD Daily
- Smoke Free
- Full Service Business Center

For more information on the Charleston Marriott Hotel Amenities, please visit us at www.marriott.com/CHSMC

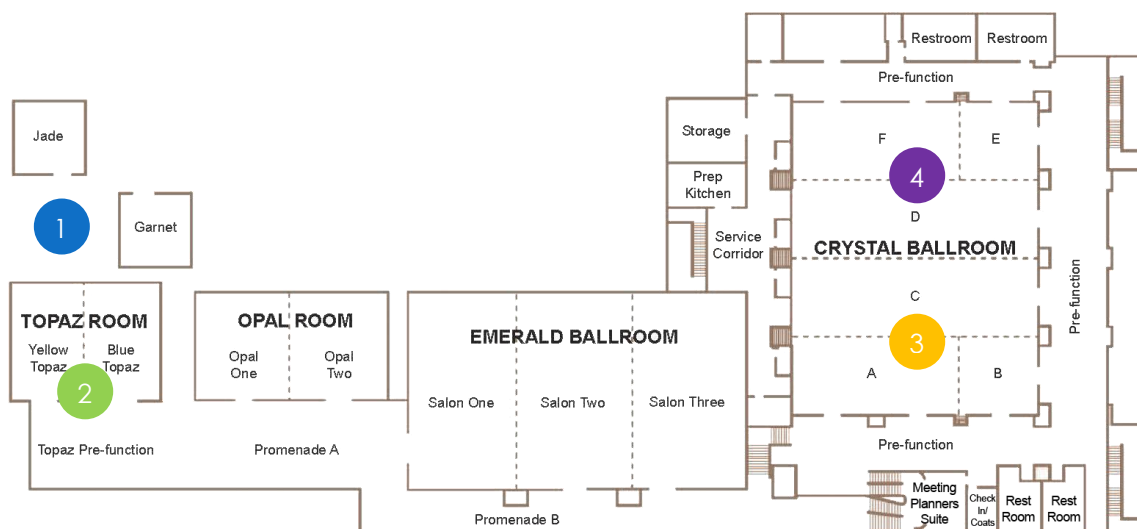
[170 Lockwood Blvd](#)
[Charleston, SC 29403](#)

Hotel Phone:
843-723-3000

Hotel Floor Plan

1 Reception will occur in Courtyard outside Topaz (in Topaz if raining)

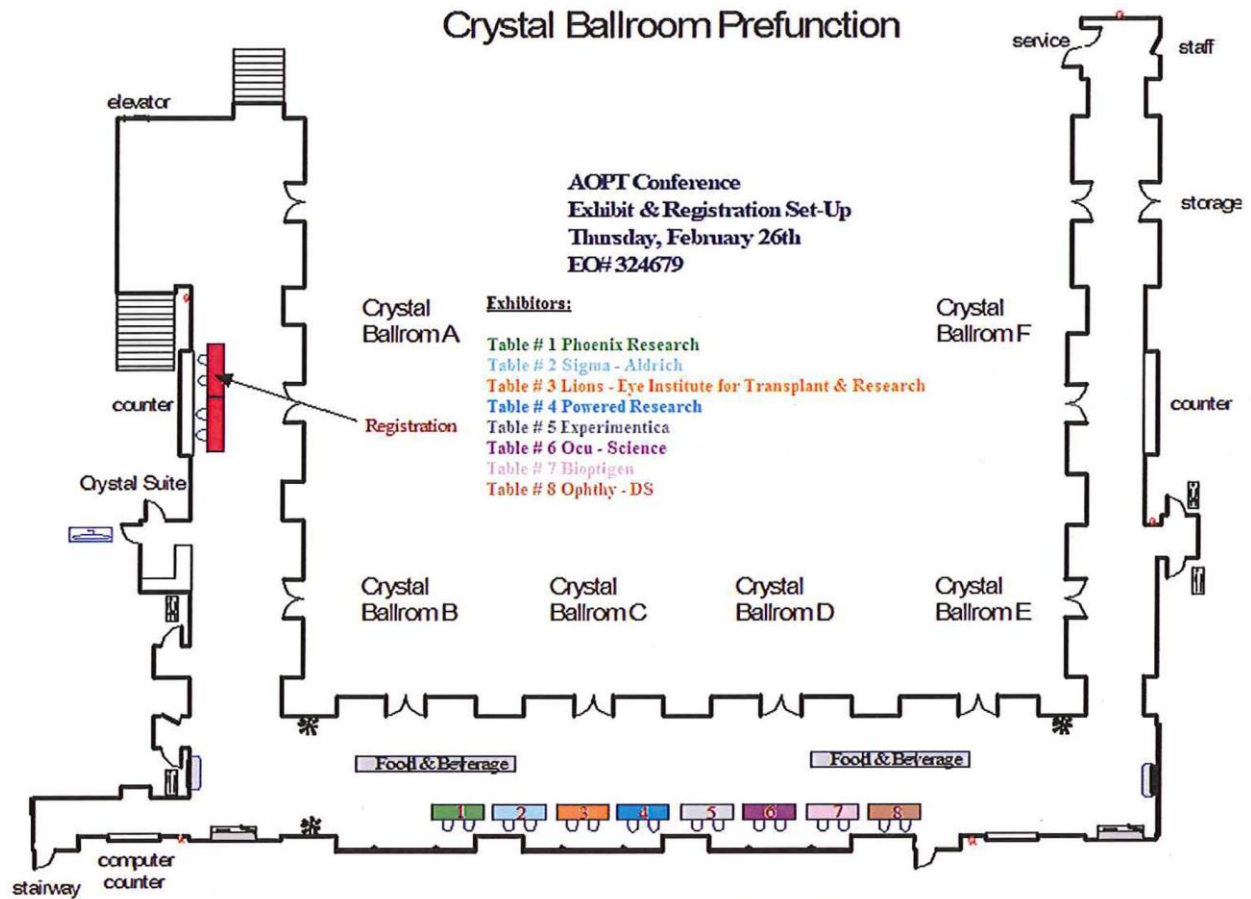
2 Banquet



4 Platform Sessions (ABC)

3 Poster Sessions (DEF)

Crystal Ballroom Prefunction



Your Program Committee



On behalf of the Program Committee (Jeff Kiel, Ganesh Prasanna and Tom Yorio), I am pleased to welcome you to Charleston, SC, for the 12th Congress on Ocular Pharmacology and Therapeutics. Our program this year is the largest in AOPT's history, with eleven sessions encompassing 57 platform presentations and a poster session with 66 presentations. We were also very fortunate to have sponsorships for 30 travel awards that bring graduate students, postdoctoral fellows and junior faculty to Charleston from all over the world. The travel award applicants were exceptional, and we are very grateful to our Awards Committee (Carol Toris, Cathy Bowes

Rickman, Julie Crider, Shahid Husain and Malinda Fitzgerald) for tackling the difficult task of selecting the finalists. Everything is lining up for an informative, interactive and productive two and a half days of cutting edge science covering all aspects of ocular pharmacology and therapeutics.

Jeff Kiel
Chair, Program Committee

Program Quick Reference

Thursday, February 26		Friday, February 27		Saturday, February 28		Sunday, March 1	
15:00 – 17:00	Registration	7:30 – 8:00	Coffee and Exhibits	7:30 – 8:00	Coffee and Exhibits	7:30 – 8:00	Coffee and Exhibits
		8:00 – 8:15	Welcome				
		8:15 – 9:15	Keynote Address: Application of systems pharmacology to retinal degenerative disorders	8:00 – 9:45	Session V Young investigators session	8:00 – 9:45	Session X Ocular ischemia and blood flow
		9:15 – 10:45	Session I Age Matters – effects of aging on ocular physiology	9:45 – 10:00	Break and exhibits	9:45 – 10:00	Break and exhibits
		10:45 – 11:00	Break and exhibits	10:00 – 11:45	Session VI Utility of intravital microscopy for ocular disease	10:00 – 11:45	Session XI Towards disease modifying drugs for treating glaucoma
		11:00 – 12:30	Session II Clinical meets pre-clinical: Translational Research	11:45 – 12:45	Lunch-n-Learn and Exhibits	11:45 – 12:00	Closing Remarks
		12:30 – 13:30	Lunch and Panel Discussion “Maximizing collaboration between academia and industry”	12:45 – 14:30	Session VII Ocular Immunology and disease		
		13:30 – 15:15	Session III AMD: clinical, pharmacological and basic science	14:30 – 14:45	Break and exhibits		
		15:15 – 15:30	Break and exhibits	14:45 – 16:30	Session VIII RNA-directed therapies for retinal diseases		
		15:30 – 17:15	Session IV Endogenous and exogenous neuroprotectors for retina	16:30 – 16:45	Break and exhibits		
17:00 – 19:15	Welcome Reception	17:15 – 17:45	AOPT General Business Meeting	16:45 – 18:30	Session IX New advances in ocular drug delivery		
		17:45 – 19:30	Poster Session, Exhibits and Reception: Hot topics poster session				
				19:00 – 21:00	Banquet Dinner		

Keynote Speaker



Krzysztof Palczewski, Ph.D

John H. Hord Professor & Chair, Department of Pharmacology

A world leader in vision research, Dr. Palczewski is best known for solving the crystal structure of the visual protein, rhodopsin. This seminal advance is especially notable because rhodopsin is now the model for understanding how this large family called G protein-coupled receptors (GPCRs) respond to various cellular signals, including hormones. Indeed, the wide distribution of GPCRs and their roles in a broad spectrum of physiological functions implicated in various disease states has made them the primary target for new drug discovery. Dr. Palczewski's latest translational research shows promise for preventing/arresting age-related macular degeneration, the major cause of human blindness.

Education

Graduated from
Gymnasium II,
Wroclaw, Poland

M.S. in Organic Chemistry
University of Wroclaw

Ph.D. in Biochemistry,
Technical University of
Wroclaw

Postdoctoral research in
ophthalmology
University of Florida,
Gainesville.

Faculty Positions
University of Florida

Oregon University of
Health Sciences, Portland

University of Washington,
Seattle, E.K. Bishop
Professor of
Ophthalmology
Pharmacology and
Chemistry

Recipient of several national/international honors and supported by multiple competitive National Institutes of Health awards and industrial grants, Dr. Palczewski and his team of 20 postdoctoral fellows and Ph. D. candidates have focused their research on the biochemistry of vision. Their investigations involve the characteristics, discovery and therapeutic manipulation of biological molecules in the retina of the eye needed for phototransduction, namely the translation of light into biochemical signals required for vision. Notable advances since Dr. Palczewski's arrival late in 2005 include:

- Advancing the Department to a ranking of 9th in the Nation. Establish the Case Center for Membrane and Structural Biology, one of the most productive centers in the country.
 - Establishing exemplary mentoring programs for junior faculty members, postdoctoral fellows and students in SOM.
 - Establishment of innovative research collaborations, consortia and joint faculty recruiting endeavors with the Departments of Ophthalmology, Otolaryngology and the School of Engineering. These initiatives enhance institutional competence in multidisciplinary research needed for major discoveries.
 - Improved capability for technology transfer. Dr. Palczewski holds several patents approved or pending and has established productive scientific relationships with industry.
- Enhanced educational capabilities. As a distinguished scientist in his field with an extensive publication record and worldwide collaborations, Dr. Palczewski adds much to our educational environment. Notably, he has enhanced our basic, interdisciplinary, and clinical research programs, aided in research training and benefited our integrated, problem-based medical curriculum through bench-based training and individual mentoring and consultation.

Major awards: Trustee Award-Foundation Fighting Blindness-2000; Alcon Research Institute-2001; Tom and Sandy Trudell Research Award-Foundation Fighting Blindness-2007; Knight's Cross of the Order of Merit-Republic of Poland, September 2011; Roger H. Johnson Macular Degeneration Award, University of Washington, June, 2012; 2012 Award-Foundation for Polish Sciences (highest ranking research award in Poland; CWRU Distinguished Researcher Award, April 2013; Friedenwald Award, ARVO, 2014; Arnold and Mabel Beckman Foundation, 2014.

Friday, February 27

Coffee and exhibits 7:30 – 8:00 a.m.

Welcome and Opening Remarks 8:00 – 8:15 a.m.

Keynote Address 8:15 – 9:15 a.m.

Application of systems pharmacology to retinal degenerative disorders

(Krzysztof Palczewski)

Session 1 9:15 – 10:45 a.m.

Age Matters - effects of aging on ocular physiology

(Carol Toris, Vikas Gulati)

Anat Galor: Aging and dry eye

Vikas Gulati and Carol Toris: Effects of Growth and Puberty on

Aqueous Humor Dynamics and Drug Response

Melissa Bailey: Ciliary muscle movement and age

Massimo A Fazio: Scleral Stiffness Changes with Age and Race in Human Eyes

Lejla Vajzovoc: Human foveal development

Break and exhibits 10:45 – 11:00 a.m.

Session 2 11:00 – 12:30 p.m.

Clinical meets pre-clinical: Translational Research

(Oliver Zeitz, Peter Kador)

Casey Kopczynski: Discovery and development of AR-13324, a first-in-class drug for glaucoma

Nanna Junker: Therapeutic innovation in diabetic retinopathy:

Developing predictive drug discovery approaches

Guido Hartmann: Characterisation of a crossmab antibody targeting VEGF and

Ang2 for neovascular diseases of the eye

Peter Kador: Nutraceutical Optixcare Eye Health Ameliorates Ocular Oxidative Stress and Maintains Tear Flow

Oliver Zeitz: Search for novel endpoints in retinology:

Public-private partnership in the IMI-2 program
Carol Toris: Consensus statements to aid in the design and implementation of translational research studies of glaucoma

Lunch Panel *12:30 – 1:30 p.m.*

Maximizing collaboration between academia and industry

(Achim Krauss, Tom Yorio)

Panelists academia: Krzysztof Palczewski (Case Western Reserve University); Leopold Schmetterer (Medizinische Universität Wien)

Panelists industry: Dennis Rice (Novartis); David Woodward (Jenivision, formerly Allergan)

Session 3 *1:30 – 3:15 p.m.*

AMD: clinical, pharmacological and basic science

(Malinda Fitzgerald, Janet Blanks)

Zsolt Ablonczy: The molecular composition of Bruch's membrane predicts aging and age-related macular degeneration

Janet Blanks: Novel Treatment for Neovascularization in Retinal Disease

Baerbel Rohrer: Local production of the alternative pathway component, factor B, is sufficient to promote laser-induced choroidal neovascularization

Matthew Campbell: Tolerability and efficacy of IL-18 in a non-human primate model of neovascular AMD

Goldis Malek: Investigating the therapeutic potential of targeting the aryl hydrocarbon receptor (AhR) in age-related macular degeneration (AMD)

Break and exhibits *3:15 – 3:30 p.m.*

Session 4 *3:30 – 5:15 p.m.*

Endogenous and exogenous neuroprotectors for retina

(Iok-Hou Pang, Dongfeng Chen)

Kip Connor: Innate Immune Mediators Facilitate Photoreceptor Cell Death in Retinal Detachment

Dong Feng Chen: A novel binding protein of insulin-like growth factor for optic nerve injury

Iok-Hou Pang: Involvement of caspase-7 in optic nerve crush-induced retinal ganglion cell death

Dennis S Rice & Nicolas G Bazan: Deletion of adiponectin receptor 1 causes photoreceptor dysfunction and degeneration

Hongli Wu: Novel Strategy for RPE Protection: Glutaredoxin-Targeting Natural Products

Petr Baranov: A novel neuroprotective small molecule for GDNF induction and photoreceptor rescue

AOPT General Business Meeting 5:15 - 5:45 p.m.

Hot Topics Poster Session 5:45 - 7:30 p.m.

Juana Gallar, Dan Stamer

Saturday, February 28

Coffee and exhibits 7:30 – 8:00 a.m.

Session 5 8:00 – 9:45 a.m.

Young investigators session

(Chris McGahan, Tom Yorio)

Teresia Carreon: Direct interaction of cochlin with mechanosensing channel TREK-1 in intraocular pressure regulation

Cula Dautriche: Evaluation of a Bioengineered Conventional Outflow Tract as a Model for High-throughput Glaucoma Drug Screening

Mike Dismuke: Trabecular Meshwork Exosomes: Role in Cellular Uptake of ECM

Karen Torrejon: Pharmacological Responsive Bioengineered 3D Human Trabecular Meshwork

Rania Sulaiman: An anti-angiogenic small molecule therapy for choroidal neovascularization

Sumana Chintalapudi: Identification of NA3 glycan as a potential candidate for atrophic AMD by promoting the development of photoreceptor outer segment assembly via receptor ligation in Muller cells

Break and exhibits 9:45 – 10:00 a.m.

Session 6 10:00 – 11:45 a.m.

Utility of intravital microscopy for ocular disease

(Pedram Hamrah, Beatrice Peebo)

Rudolff Guthoff: Nerve fiber density in the corneal subbasal plexus, its role in the follow-up of diabetic neuropathy

Pedram Hamrah: Utility of in vivo confocal microscopy-based imaging endpoints for the assessment of ocular surface inflammation

Edoardo Villani: In vivo confocal microscopy of the ocular surface in dry eye: over the cornea

Neil Lagali: Towards development of improved anti-angiogenic therapies: insights from in vivo confocal microscopy in the murine cornea

Beatrice Peebo: Imaging models for assessing angiogenesis and evaluating anti-angiogenic therapies

Lunch-n-Learn tables 11:45 – 12:45 p.m.

Meet the Keynote Speaker – *Krzysztof Palczewski*

Relocation – *Carol Toris*

Academic Career – *Thomas Yorio, Juana Gallar*

MD/PhD – *Shahid Husain, Filippo Drago*

Transition Academia - Industry – *Ganesh Prasanna*

Balancing Act: family, research and heavy teaching – *Malinda Fitzgerald*

Industry Career – *Achim Krauss, Oliver Zeitz*

Session 7 12:45 – 2:30 p.m.

Ocular Immunology and disease

(Rachel Caspi, Heping Xu)

Daniel Saban: Immune responses at the ocular surface

Rachel Caspi: Ocular Autoimmunity – a collusion of development and environment

Shikhar Mehrotra: T Cells in Ocular Dysfunction:

Lessons From a Vitiligo Prone Novel Transgenic Mouse

Heping Xu: Immunology of retinal degenerative disease

Break and exhibits 2:30 – 2:45 p.m.

Session 8 2:45 – 4:30 p.m.

RNA-directed therapies for retinal diseases

(Ash Jayagopal, Shusheng Wang)

Alfred Lewin: RNA Based Gene Therapy for Autosomal Dominant Retinitis Pigmentosa

Shunbin Xu: miRNAs in diabetic retinopathy and treatment

Ashwath Jayagopal: Targeted Nanocarriers for Therapy of Retinal Vascular Disease

Shusheng Wang: Noncoding RNAs in ocular angiogenesis

Xavier Gérard: AON intravitreal injections to manipulate splicing in retinal cells

Break and exhibits 4:30 – 4:45 p.m.

Session 9 4:45 – 6:30 p.m.

New advances in ocular drug delivery

(Uday Kompella, Cheryl Rowe-Rendleman)

Bryce Chiang: The Suprachoroidal Space Accessed by Microneedle Injection

Michael Robinson: Pharmacokinetic/ Pharmacodynamic Considerations with Ocular Drug Delivery Implants

Steve Giannos: Photokinetic Drug Delivery:
Light-enhanced Permeation In An In Vitro Model Eye
Kim Brazzell: Nanoparticles in Ocular Drug Delivery
Morgan V. Fedorchak: The Monthly Eye Drop: Preclinical testing of long-term,
hydrogel/microsphere eye drops for glaucoma

Banquet Dinner 7:00 – 9:00 p.m.

Sunday, March 1

Coffee and exhibits 7:30 – 8:00 a.m.

Session 10 8:00 – 9:45 a.m.

Ocular ischemia and blood flow

(Jeff Kiel, Doreen Schmidl)

Crawford Downs: Perfusion pressure: continuous telemetry measurement of blood pressure and bilateral IOP in primates

Leo Schmetterer: Oxygen modulation of flicker-induced blood flow changes in the human retina

Doreen Schmidl: Retinal oxygen metabolism in healthy subjects

Taiji Nagaoka: Role of Neuronal Nitric Oxide Synthase in Regulating Retinal Blood Flow In Response to Flicker-Induced Hyperemia in Cats

Jeff Kiel: AR-13324 lowers EVP in Dutch Belted rabbits

Break and exhibits 9:45 – 10:00 a.m.

Session 11 10:00 – 11:45 a.m.

Towards disease modifying drugs for treating glaucoma

(Jenny Wang, David Woodward)

Abbot Clark: Retinal ganglion cell neuroprotection by JNK inhibition and neuritin-1 gene therapy

Terete Borrás: Predictable candidate genes for glaucoma gene therapy

Jenny Wang: Disease Modifying/ Reversing Anti-Glaucoma Drugs: A High-Throughput Method for Lead Discovery **Rama Krishnan:** Novel antiglaucoma therapies that target endothelial cell contraction

David Woodward: Steroid-Induced Glaucoma in Mice: A Model with Potential for Studying Disease Modification /Reversal

Closing Remarks 11:45 a.m.

Platform Abstracts

Aging and dry eye

Anat Galor^{1, 2},

¹*Ophthalmology, Miami VAMC, Miami, FL;*

²*Bascom Palmer Eye Institute, University of Miami, Miami FL.*

Purpose: To discuss likely etiologies that underlie the association between aging and dry eye.

Methods: Review of the literature

Results: Changes in the lacrimal gland (producer of the aqueous component of tears), meibomian glands (producers of the lipid component) and goblet cells (producers of the mucin component) have all been described with aging and may explain why older individuals have less healthy tear parameters than younger patients. Inflammation is a well-recognized component of dry eye and in human, T cells have been found in the conjunctiva and inflammatory cytokines in tears. Older mice have been noted to develop this phenotype spontaneously and this finding may mirror the known immune dysregulation that can occur with age. Furthermore, aging also likely impacts the function of the ocular sensory apparatus (both peripheral and central changes) which may explain the higher frequency of dry eye symptoms in the older compared to younger population.

Conclusions: Multiple factors likely underlie the noted association between aging and dry eye including changes in anatomic structures associated with dry eye, an increased propensity for ocular surface inflammation, and sensitization of the ocular sensory apparatus.

Effects of Growth and Puberty on Aqueous Humor Dynamics and Drug Use

Vikas Gulati¹, Cassandra L Hays¹, Shan Fan¹, Shane J Havens¹, **Carol B Toris**^{1,2}

¹*Truhlsen Eye Institute, University of Nebraska Medical Center, Omaha NE,*

²*Case Western Reserve University*

Purpose: To identify the effects of growth and puberty on aqueous humor dynamics (AHD) and changes therein in response to intraocular pressure (IOP) lowering medications.

Methods: Biometric and AHD data were collected longitudinally from male Dutch-Belted rabbits between ages 9 and 42 weeks. Body weight, testicular volume, and serum testosterone were measured to monitor development. Variables studied included anterior chamber depth and volume, corneal thickness, intraocular pressure (IOP), aqueous flow, episcleral venous pressure (EVP) and outflow facility. Systemic acetazolamide or topical timolol, latanoprost, or saline (control) were administered pre and post-puberty to assess their effects on IOP.

Results: Body weight, testicular volume, and serum testosterone increased to stabilize at 28 weeks. IOP increased until week 18, then decreased through week 26 followed by another increase. Aqueous flow peaked at 14 weeks at 2.42 ± 0.90 $\mu\text{l}/\text{min}$ followed by a decrease at 30 weeks to 1.91 ± 0.80 $\mu\text{l}/\text{min}$ (2 way RMANOVA $p=0.02$). Tonographic outflow facility did not significantly change throughout development ($p=0.97$). EVP was the lowest at 10 weeks (7.82 ± 1.32 mmHg) and highest at 22 weeks (14.49 ± 2.64 mmHg; $p<0.0001$). Timolol, latanoprost, or acetazolamide decreased IOP post-puberty but not pre-puberty.

Conclusions: The initial IOP increase in pre-pubertal rabbits coincides with increasing body weight and a period of increasing EVP. The IOP trough at 26 weeks could not be explained by changes in aqueous flow, outflow facility or EVP which suggests development of and increased flow through the uveoscleral pathway. IOP lowering medications were effective only post puberty when eye development stabilized.

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Ciliary muscle movement and age

Melissa D Bailey, OD, PhD¹, *The Ohio State University, College of Optometry, Columbus, USA*

Purpose: To describe changes in ciliary muscle thickness with age and accommodation.

Methods: In cross-sectional studies, the ciliary muscle was imaged with anterior segment optical coherence tomography (AS-OCT), and thickness was measured along the length of the muscle {maximum thickness (CMTMAX), and 1 mm (CMT1), 2mm (CMT2), and 3mm (CMT3) posterior to the scleral spur}. The forward movement of the ciliary muscle apex was also measured. The muscle was imaged at rest in 778 subjects (ages 3-85 years) at rest and during 4-D of accommodation in 23 subjects (ages 18-30 years).

Results: A 4.0 D change in accommodation increased CMTMAX by $78.09 \pm 64.80 \mu\text{m}$ ($p < 0.0001$) and CMT1 $69.25 \pm 61.37 \mu\text{m}$ ($p < 0.0001$), and the ciliary muscle apex moved forward by $156.57 \pm 265.03 \mu\text{m}$ ($p = 0.009$). Across all ages, the relationship between anterior CMT and age was quadratic (CMT1, intercept = 713.4, Age: $\beta=6.8$, $p=0.00001$, Age²: $\beta=-0.17$, $p=0.0004$, AgeXAge²: $\beta=0.001$, $p=0.003$). Substantial increases in thickness were found across subjects in the first decade of life (CMTMAX: $\beta=2.7$, $p=0.01$, CMT1: $\beta=10.6$, $p=0.00002$, CMT2: $\beta=7.8$, $p=0.0002$, CMT3: $\beta=9.0$, $p=0.00002$).

Conclusions: In the first decade of life, younger children have substantially thinner ciliary muscles than older children. This thickness then appears to plateau in subjects in early adulthood, but is then slightly thinner again in subjects in the latter decades of life. AS-OCT is capable of documenting changes in thickness with accommodation, where the anterior region of the muscle moves forward and inward during accommodation.

Scleral Stiffness Changes with Age and Race in Human Eyes

MA Fazio^{1,2}, R Grytz¹, JS Morris³, L Bruno², CA Girkin¹, JC Downs¹

¹Ophthalmology, University of Alabama at Birmingham Medical Center, Birmingham, AL.

²Mechanical Engineering, University of Calabria, Italy.

³Department of Biostatistics, The University of Texas MD Anderson Cancer Center, Houston, Texas;

Purpose: To test the hypothesis that the variation of peripapillary scleral structural stiffness with age is different in donors of European (ED) and African descent (AD)

Methods: Twenty-nine pairs of normal eyes from human donors (9 AD, 20 ED) aged 0 to 90 years old were mechanically inflation tested within 48 hours post mortem as follows. The intact posterior scleral shell of each eye was pressurized from 5 to 45 mmHg while the full-field three-dimensional displacements of the scleral surface were measured using laser speckle interferometry. Mean maximum principal (tensile) strain was computed within the peripapillary and mid-peripheral regions surrounding the optic nerve head (ONH). The peripapillary and mid-peripheral regions were defined as a ~9 degree-wide-band adjacent to the ONH and the band of equal surface area immediately outside the peripapillary region, respectively.

Results: Mechanical tensile strain significantly decreased with age in the peripapillary sclera in both the African and European descent groups ($P < 0.001$), but the age-related stiffening was significantly greater in the African descent group ($P < 0.05$).

Conclusions: These results indicate 1) the peripapillary sclera is subjected to significantly higher tensile strain than the mid-peripheral sclera, independent of race, 2) AD eyes showed a more rapid stiffening with age than ED eyes, and 3) while the mid-peripheral region doesn't significantly stiffen with age in the ED eyes, a rapid and significant stiffening of the mid-peripheral sclera is present in the AD eyes. These significant racial differences in scleral biomechanics may contribute to the increased susceptibility of AD persons to glaucoma.

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Human Foveal Development

Lejla Vajzovic¹, Anita E. Hendrickson², Rachelle V. O'Connell¹, Laura A. Clark¹, Du Tran-Viet¹, Daniel Possin², Stephanie J. Chiu³, Sina Farsiu^{1,3}, Cynthia A. Toth^{1,3}

¹*Department of Ophthalmology, Duke University Eye Center, Durham, North Carolina.*

²*Department of Biological Structure, Seattle, Washington; Department of Ophthalmology, University of Washington, Seattle, Washington.*

³*Department of Biomedical Engineering, Pratt School of Engineering, Durham, North Carolina. Cynthia A. Toth, Department of Ophthalmology, Duke University Eye Center, Durham, North Carolina; Department of Biomedical Engineering, Pratt School of Engineering, Durham, North Carolina.*

Purpose: To describe the human foveal development from late gestation to adulthood.

Methods: Morphology and layer thickness of retinal Spectral Domain Optical Coherence Tomography (SDOCT) images from premature and term infants, children, and adults without macular disease were compared to light microscopic histology from comparable ages.

Results: SDOCT images correlate with major histologic findings at all time points. With both methods, preterm infants demonstrate a shallow foveal pit indenting inner retinal layers (IRL) and short, undeveloped foveal photoreceptors. At term, further IRL displacement forms the pit and peripheral photoreceptors lengthen; the elongation of inner and outer segments (IS and OS, histology) separates the IS band from retinal pigment epithelium. Foveal IS and OS are shorter than peripheral for weeks after birth (both methods). By 13 months, foveal cone cell bodies stack >6 deep, Henle fiber layer (HFL) thickens, and IS/OS length equals peripheral; on SDOCT, foveal outer nuclear layer (which includes HFL) and IS/OS thickens. At 13 to 16 years, the fovea is fully developed with a full complement of SDOCT bands; cone cell bodies >10 deep have thin, elongated, and tightly packed IS/OS.

Conclusions: We provide qualitative and quantitative morphological information necessary to understand the marked changes in human retinal development from late gestation to adulthood.

Discovery and development of AR-13324, a first-in-class drug for glaucoma

Casey Kopczynski, PhD, *Aerie Pharmaceuticals, Inc., Research Triangle Park, NC*

Purpose: To provide a “bench to bedside” overview of the development of AR-13324, the first of a new class of ocular hypotensive drugs that inhibit both Rho kinase (ROCK) and the norepinephrine transporter (NET). AR-13324 is currently in Phase 3 clinical trials for lowering IOP in patients with open-angle glaucoma and ocular hypertension.

Methods: Medicinal chemistry, kinase profiling and high content cell-based screening were used to characterize over 1,500 newly synthesized ROCK inhibitors. Preclinical efficacy and mechanism of action studies were conducted in rabbits and monkeys. Clinical trials were double-masked and randomized, and included active comparators.

Results: AR-13324 inhibits both ROCK and NET. It lowers IOP in animal models by increasing outflow facility, decreasing aqueous production, and lowering episcleral venous pressure.

In a Phase 2b clinical trial, once-daily AR-13324 reduced mean IOP by 5.7 – 6.2 mmHg and was well tolerated, with a 24% incidence of mild to moderate hyperemia on Day 28. AR-13324 was less effective than latanoprost in high baseline patients, but equally as effective as latanoprost in patients with moderately elevated IOPs.

PG324, a fixed-dose combination of AR-13324 and latanoprost, produced superior IOP lowering relative to both latanoprost and AR-13324 in a 28-day Phase 2b trial. The addition of latanoprost to AR-13324 did not increase the incidence of hyperemia adverse events beyond that of AR-13324 alone.

Conclusions: AR-13324 provides new mechanisms of action for lowering IOP. It appears highly effective and well-tolerated in patients, both as monotherapy and in combination with latanoprost.

Therapeutic innovation in diabetic retinopathy: Developing predictive drug discovery approaches

Nanna Junker, Frederik W. Sand, Andrea Lundqvist, Anker J. Hansen, and Daniel B. Timmermann *Diabetic Complications, Novo Nordisk, Novo Nordisk Park, Maaloev, Denmark*

Diabetic retinopathy (DR) is the leading cause of vision loss among the working age population in the Western world. The pathogenesis involves changes in metabolic parameters affecting the retinal vasculature leading to increased vascular permeability and neovascularization. Ultimately, the changes can progress into vision threatening diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR).

Predictive drug discovery approaches depend on in vivo and cellular experimental setups that reflect key pathological mechanisms. Rodent models are popular in drug discovery because of size and accessibility. However, it is well recognized that rodent models of DR reflect only early changes in DR, similar to non-proliferative DR, but fail to progress to stages reflecting DME and PDR. Thus, alternative short and predictive mechanistic animal models reflecting key pathological processes are both useful and necessary. When using non diabetic mechanistic models it is of profound importance to recognize confounding pathological processes in the surrogate models and to understand diabetic disease driven parameters that are not reflected. Predictive cellular mode of action models and in vitro bioassays allowing in depth evaluation of target biology can complement and enhance the understanding of the pathological processes affected throughout the disease as well as the mechanism of proposed drug targets. To develop predictive drug discovery and therapeutic innovation within diabetic retinopathy a mechanistic focus in both animal and cellular experimental approaches is necessary to complement each other when validating future drugs for patients suffering from DR.

Characterisation of a crossmab antibody targeting VEGF and Ang2 for neovascular diseases of the eye

Guido Hartmann, PhD, *Neurology Ophthalmology and Rare Disease DTA, Roche Pharma Research and Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd*

Age related macular degeneration, retinal vein occlusion and diabetic macular edema are among the major causes of loss of vision in developed countries. Pathological neovascularization and vessel leakiness are key mechanisms contributing to function loss in the retina. Anti-VEGF-A therapy has become the main therapy option in the clinic. To investigate the possibility to enhance the efficacy of anti-VEGF-A therapy further, we tested the combination of anti-VEGF-A antibodies with the inhibition of another key angiogenic factor, namely angiopoietin-2 (ANG-2) in preclinical models of eye disease. Ang-2 is an antagonist/context dependent partial agonist of the Tie-2 receptor. ANG-1, the second ligand of Tie-2 receptor tyrosine kinase keeps endothelial cells in a normalized state and protects from proangiogenic signaling. In contrast, increased levels of ANG-2 make vessels receptive to proinflammatory and proangiogenic signaling. Indeed, anti-ANG-2 treatment reduces the degree of choroidal neovascularization and vessel leakiness in a mouse model of spontaneous choroidal neovascularization. Combined treatment with VEGF and ANG2 antibodies showed enhanced efficacy compared to each treatment alone. RG7716 is a fully human crossmab immunoglobulin antibody that is capable of simultaneously binding VEGF-A with one Fab arm and ANG2 with the other Fab arm. High affinity and simultaneous binding was demonstrated for both ligands. Efficacy of crossmab antibody RG7716 was tested in the laser-induced model of choroidal neovascularisation in nonhuman primates.

Nutraceutical Optixcare Eye Health Ameliorates Ocular Oxidative Stress and Maintains TearFlow

Peter F Kador^{1,2}, Changmei Guo¹, Hiroyoshi Kawada¹, James Randazzo¹, and Karen Blessing^{1,2}

¹*Department of Pharmaceutical Sciences University of Nebraska Medical Center, Omaha, NE, USA*

²*Therapeutic Vision Inc., Omaha, NE, USA*

Purpose: Targeting oxidative pathways associated with age-related lens, retinal and tear flow diseases suggest therapeutic potential. Based on the hypothesis that oral nutraceuticals may not adequately reach ocular tissues, particularly those in the anterior segment, we have evaluated the ability of a topical nutraceutical antioxidant formulation to ameliorate oxidative stress in three rat models of these age-related ocular complications.

Methods: Oxidative stress was induced in rats by 15 min exposure to 1600 $\mu\text{W}/\text{cm}^2$ of UV light, diabetes by tail vein injection of streptozotocin, and exposure of dark adapted rats to 1000 lx of light. Dry eye was induced in rats by b.i.d. subcutaneous scopolamine injections.

Results: In rats exposed to UV light, Optixcare EH was able to significantly protect lenses against decreased GSH levels induced by exposure to UV light. Similarly, these treatments were able to significantly delay the progression of cataracts induced by diabetes. In light damaged rats, Optixcare EH partially protected the neural retina and photoreceptors against oxidative stress. Optixcare EH also maintained tear flow in the scopolamine treated rats.

Conclusion: Topical administration of this nutraceutical can reduce experimentally induced ocular oxidative stress in rats exposed to several sources of ocular oxidative stress. This suggests that the ingredients in Optixcare Eye Health are able to reach not only the anterior segment but also partially the posterior segment following topical administration. Planned open label clinical studies will evaluate the beneficial effects observed for the treatment of dry eye.

Search for Novel Endpoints in Retinology: Public-Private Partnership in the IMI-2 Program

Oliver Zeitz, *Global Clinical Development Ophthalmology, Bayer Pharma AG, Berlin, Germany*

Purpose: Dry AMD and Diabetic Retinopathy are common eye diseases. Both typically evolve chronically with no immediate vision impairment. However, over time sight threatening events accumulate after which vision can often not be restored with currently available treatments. There is a lack of validated parameters, which would predict the probability of such events and hence would qualify as study endpoints.

Methods: Under the umbrella of the Innovative Medicines Initiative, a public-private partnership between EU and EFPIA, a project has been initiated to discover and validate novel endpoints in dry AMD and diabetic retinopathy.

Results: The industry consortium has been joined by 6 pharma companies and one imaging company. The aim of this 5-year project is to evaluate novel endpoint candidates for dry AMD and DR for use in clinical trials investigating drug or other therapies. The evaluation will cover the technical, medical and health economic appropriateness of a method and bridge preclinical and clinical studies. The presentation will discuss the medical/scientific need and will update on the status of the program.

Conclusions: Collaborative research is required to pave the way for successful future drug development in ophthalmology and to address remaining unmet medical needs.

Consensus statements to aid in the design and implementation of translational research studies of glaucoma

Carol B. Toris¹ and the glaucoma research advisory group, *¹Department of Ophthalmology, Case Western Reserve University, Cleveland, OH*

The Association for Ocular Pharmacology and Therapeutics has established an initiative to develop definitive guidelines for designing, conducting and analyzing translational research studies. This is a report of the preliminary activities of the glaucoma research advisory group composed of glaucoma basic scientists and clinicians from industry and academia tasked with writing the glaucoma consensus statements. These statements will identify the key steps needed before starting a study, during study progression and after study completion. Pre-study choices include the best pre-clinical models, predictive features, effect sizes, study design, endpoints, meaningful information and proof of concept. During the study, judgments maybe required on whether or when to change the protocol or terminate a study prematurely. At study completion, decisions are needed on statistical tests, handling missing data points, addressing new questions, recognizing key findings, and designing the next study. When completed, the consensus statements will aid in determining the best designs to answer the pivotal questions to move an effective product quickly and safely into clinical trials.

The molecular composition of Bruch's membrane predicts aging and age-related macular degeneration

Zsolt Ablonczy (Presenter), Hannah E. Bowrey, E. Ellen Jones, Mark A. Fields, Lucian V. Del Priore, Rosalie K. Crouch. *Department of Ophthalmology, Medical University of South Carolina, Charleston, SC, USA.*

Purpose: Bruch's membrane (BM) gradually deteriorates with aging and a significant amount of drusen accumulates between the retinal pigment epithelium (RPE) and BM. Large soft drusen in the posterior pole is the basis of clinical age-related macular degeneration (AMD) diagnosis; however, the significance of drusen in AMD development is not understood.

Methods: Here, we utilized a multimodal molecular imaging approach to examine flatmounted human BMs (n = 15, ages 21-88) at 350 μm resolution. The molecules underlying drusen were determined from co-registering the fluorescence images with individual molecules collected from the same tissue. Tissue with no relevant ocular history were compared to AMD patients using principal component analysis (PCA) to identify specific molecular patterns in BMs.

Results: 500 molecules per sample exhibited significant spatial correlation with BM autofluorescence and 311 molecules correlated with age. However, only three molecules (m/z 268, 410, and 426) increased with age ($R = 0.77, 0.78$ and 0.75 , respectively; $p < 0.05$), while other molecules decreased with age. A subset of lipophilic molecules correlating with lesions were present in AMD tissue only but bisretinoids were not found in significant quantities. PCA analysis distinguished BMs of different ages and disease states.

Conclusions: Multimodal molecular imaging of BM could reliably predict the clinical diagnosis of the tissue. Age-related changes were associated with the loss/conversion of many small molecules but the accumulation of only three molecules suggests an importance in AMD pathobiology. These experiments link diagnostically relevant clinical features to the underlying molecules in AMD.

Novel Treatment for Neovascularization in Retinal Disease

Janet C. Blanks¹, Manas R. Biswal², C. Kathleen Dorey³, and Howard M. Prentice⁴

¹Center for Complex Systems and Brain Sciences, Florida Atlantic University, Boca Raton, FL,

²Molecular Genetics and Microbiology, College of Medicine, University of Florida, Gainesville, FL,

³Virginia Tech Carilion School of Medicine, Roanoke, VA, ⁴Biomedical Sciences, College of Medicine, Florida Atlantic University, Boca Raton, FL

Purpose: Neovascularization in age-related macular degeneration and diabetic retinopathy remains a major cause of vision loss. Müller cells in the retina, play a significant role in retinal neovascularization (NV) in response to tissue hypoxia. We previously designed and tested a vector using a hypoxia-responsive domain and a glial fibrillary acidic protein (GFAP) promoter to drive GFP expression in Müller cells in the murine model of oxygen-induced retinopathy (OIR). This study compares the efficacy of regulated and unregulated Müller cell delivery of endostatin in preventing NV in the OIR model.

Methods: Endostatin cDNA was cloned into plasmids with hypoxia regulated GFAP or unregulated GFAP promoters, and packaged into self complementary AAV2 vectors (scAAV2). Before placement in hyperoxia on day 7, mice were given intravitreal injections of regulated or unregulated scAAV2, capsid, or PBS. On day 17, five days after return to room air, neovascular and avascular areas, as well as expression of the transgene and VEGF, were compared in OIR animals treated with a vector, capsid, or PBS.

Results: The hypoxia-regulated, glial-specific vector expressing endostatin reduced NV by 93% and reduced the central vaso-obliteration area by 90%, matching the results with the unregulated GFAP-Endo vector. Retinas treated with the regulated endostatin vector expressed substantial amounts of endostatin protein, and significantly reduced VEGF protein. Endostatin production from the regulated vector was undetectable in retinas with undamaged vasculature.

Conclusion: These findings suggest that the hypoxia-regulated, glial cell-specific vector expressing endostatin may be useful for treatment of NV in proliferative diabetic retinopathy.

Local production of the alternative pathway component, factor B, is sufficient to promote laser-induced choroidal neovascularization.

Bärbel Rohrer^{1,2}, Gloriane Schnabolk¹, Beth Coughlin², Kusumam Joseph², Elizabeth C. O'Quinn², Mausumi Bandyopadhyay², Kannan Kunchithapautham², Tamara Nowling³

¹Research Service, Ralph H. Johnson VA Medical Center, Charleston, SC 29401. ²Departments of Ophthalmology, and Medicine, Division of Rheumatology and Immunology³, Medical University of South Carolina, Charleston, SC 29425.

Purpose: Complement factor B (CFB) is a required component of the alternative pathway (AP) of complement; and CFB polymorphisms are associated with age-related macular degeneration (AMD) risk. CFB is made in the liver, but expression has also been detected in retina and retinal pigment epithelium (RPE)-choroid. We investigated whether production of CFB by the RPE can promote AP activation in mouse choroidal neovascularization (CNV).

Methods: Transgenic mice expressing CFB under the RPE65 promoter were generated and crossed onto factor-B-deficient (CFB-KO) mice. Biological activity was determined in vitro using RPE monolayers, and in vivo using laser-induced CNV. Contribution of systemic CFB was investigated using CFB-KO reconstituted with CFB-sufficient serum.

Results: Transgenic mice (CFB-tg) express CFB in RPE-choroid at ~15% wild type level; no CFB was detected in serum. Cultured CFB-tg RPE monolayers secrete CFB apically and basally upon exposure to oxidative stress that was biologically active. CNV sizes were comparable between wild type and CFB-tg mice, but significantly increased when compared to lesions in CFB-KO mice. Injections of CFB-sufficient serum into CFB-KO mice resulted in partial reconstitution of systemic AP activity and significantly increased CNV size.

Conclusions: Mouse RPE cells express and secrete CFB sufficient to promote RPE damage and CNV. This further supports that local complement production may regulate disease processes; however, the reconstitution experiments suggest that additional components may be sequestered from the bloodstream. Understanding the process of ocular complement production and regulation will further our understanding of the AMD disease process and the requirements of a complement-based therapeutic.

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Tolerability and efficacy of IL-18 in a non-human primate model of neovascular AMD

Matthew Campbell¹, Peter Adamson², Francisco J. López³, Edit Kurali⁴, Peter Humphries¹, Sarah L Doyle⁵

¹Ocular Genetics Unit, Smurfit Institute of Genetics, Trinity College Dublin, Dublin 2, Ireland.

²Ophthalmology Discovery Performance Unit, GlaxoSmithKline, Stevenage, United Kingdom.

³Ophthalmology Discovery Performance Unit and ⁴Statistics Consulting Group. Quantitative Science, PTS, GlaxoSmithKline, King of Prussia, PA 19406, United States.

⁵Dept. of Clinical Medicine, School of Medicine, Trinity College Dublin, Dublin 2, Ireland

Purpose: Age-related macular degeneration (AMD) is the most common form of central retinal blindness in the developed world. Activation of the NLRP3-inflammasome has recently come to the fore as being involved in the development of both “dry” and neovascular (“wet”) forms of the disease. We have previously shown that IL-18 can regulate choroidal neovascularization (CNV) formation in mice. To address the translational potential of IL-18, we initiated a major non-human primate tolerability and efficacy study for the use of intravitreally (IVT) or systemically administered clinical grade human IL-18 (SB-485232).

Methods: Cynomolgus monkeys were injected IVT with human IL-18 (2 each at 100 ng, 1000 ng, 3000 ng and 10,000 ng per eye). In addition, 26 monkeys were administered 9 laser burns in each eye prior to injection of IL-18 as an IVT or systemic bolus at a range of doses. Fundus fluorescein angiography (FFA) was performed on days 15, 22 and 29 post injection and the development of neovascular lesions was assessed.

Results: No IL-18-related ophthalmoscopic/histopathological anomalies were apparent even at a dose of 10,000 ng per eye. We now show that IL-18 can prevent laser induced CNV development in the non-human-primate eye both as an IVT or systemic injection.

Conclusions: IL-18 is safe and has efficacy in preventing laser induced CNV in the non-human primate eye. Human IL-18 is bioactive in monkeys yet does not cause RPE or retinal cell death and could now represent a novel immuno-therapeutic based monotherapy or adjunctive strategy for the treatment of neovascular AMD in human subjects.

Investigating the therapeutic potential of targeting the aryl hydrocarbon receptor (AhR) in age-related macular degeneration (AMD)

Goldis Malek^{1,2} (Presenter), Mayur Choudhary¹, David L. Forest⁴, Stephen H. Safe³ and Lincoln V. Johnson⁴

¹*Department of Ophthalmology, Duke University School of Medicine, Durham, NC*

²*Department of Pathology, Duke University School of Medicine, Durham, NC*

³*Department of Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX*

⁴*Center for the Study of Macular Degeneration, University of California, Santa Barbara, CA*

Purpose: AhR is a transcription factor responsible for toxin metabolism. We have shown that AhR activity in human retinal pigment epithelial (RPE) cells declines with age, and aged *AhR* knockout mice develop ocular features of dry AMD and exaggerated laser induced choroidal neovascular (CNV) lesions. Given the lack of effective treatments for AMD, the leading cause of vision loss in the elderly, we tested the therapeutic potential of targeting AhR, using the FDA approved drug, leflunomide, also an AhR agonist.

Methods: AhR activity and target gene expression in human RPE (ARPE19), human fetal RPE (fRPE) and rhesus macaque choroidal endothelial (RF6A) cell lines treated with leflunomide were assessed using luciferase reporter assays and qPCR. Sub-RPE ‘drusenoid’ deposit formation in the fRPE cell culture model was evaluated by light microscopy. Cell migration and endothelial tube formation were measured using a monolayer wound healing assay and GeltrexTM basement membrane matrix, respectively. Finally, the effect of AhR activation on severity of experimentally induced CNV lesions in aged C57BL/6J mice was evaluated.

Results: Leflunomide treatment resulted in AhR activation and upregulation of *AhR*-specific target genes, in both ARPE19 and RF6A cells, inhibition of collagen and fibronectin secretion, and decreased expression of monocyte chemo-attractant protein-1, in ARPE19 cells. Agonist treatment decreased *in vitro* sub-RPE deposit formation by 71% and inhibited *AhR* knockdown-induced migration and tube formation of choroidal endothelial cells.

Conclusion: Pharmacologic modulation of AhR activity may be a viable path in ameliorating pathologies associated with both early dry- and wet-AMD.

Innate Immune Mediators Facilitate Photoreceptor Cell Death in Retinal Detachment.

Kip M. Connor¹, J. Harry Sweigard¹, Hidetaka Matsumoto¹, Leo Kim²

¹ *Angiogenesis Laboratory, Department of Ophthalmology, Harvard Medical School, Massachusetts Eye & Ear Infirmary, 243 Charles Street, Boston, MA 02114, USA.*

² *Schepens Eye Research Institute, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, USA.*

Purpose: Degeneration of photoreceptors is a primary cause of vision loss worldwide making the underlying mechanisms surrounding photoreceptor cell death critical to developing new treatment strategies. Retinal detachment (RD) is a sight threatening disorder that can happen in diseases like retinopathy of prematurity, diabetic retinopathy (tractional RD), ocular tumors, and age-related macular degeneration (exudative RD). The complement system is an intricate innate immune surveillance pathway that is able to discriminate between healthy host tissue, diseased host tissue, apoptotic cells and foreign invaders while modulating the elimination and repair of host tissue accordingly. Little is known, however, about the role of the complement system in RD.

Methods: We will utilize a well-defined mouse model of RD, whereby a subretinal injection of sodium hyaluronate is used to create a detachment. The mouse RD model will allow us to take advantage of well-established genetic manipulation platforms in mice (e.g. complement deficient knock out strains).

Results: We found that photoreceptors downregulate membrane bound inhibitors of complement, allowing for their selective targeting by the complement system. When the photoreceptors in the detached retina are removed from the primary source of oxygen and nutrients (choroidal vascular bed) the retina becomes hypoxic leading to an upregulation of complement. Preventing complement production using knockout mice or through pharmacologic inhibition ameliorates much of the photoreceptor cell death.

Conclusions: Understanding the mechanism by which the innate immune system facilitates photoreceptor cell death will provide significant therapeutic insight in our management of this pathology and other neurodegenerative conditions.

A novel binding protein of insulin-like growth factor for optic nerve injury

Dong Feng Chen^{1,2} (Presenter), Chenying Guo¹, Kin-Sang Cho¹, Kissauo Tchedre¹

¹Schepens Eye Research Institute, Massachusetts Eye and Ear, Department of Ophthalmology, Harvard Medical School, Boston, MA 02114;

²Boston VA Healthcare System, 150 S. Huntington Ave, Boston, MA 02130

Purpose: At a certain point in mammalian development, neurons in the central nervous system lose their ability to grow axons, which leads to catastrophic consequence and permanent functional deficit after injury. Activation of axonal growth program is a critical step in successful nerve regeneration following injury. Yet the molecular mechanisms that orchestrate this transition are not fully understood.

Methods: Using immunohistochemistry and real time polymerase chain reaction, we examine the expression of insulin-like growth factor 1 (IGF-1), IGF-1 binding protein-like 1 (IGFBPL1), and IGF-1 receptor in retinal ganglion cell (RGC) during development. Using shRNA gene knockdown and mouse genetic engineering technology, we investigated the role for IGFBPL1 in RGC survival and axon growth in vitro and in vivo. Moreover, IGFBPL1 was added to purified RGC cultures or administered by intravitreal injection following optic nerve crush injury in vivo.

Results: Here we report the identification of an essential regulator IGFBPL1 that in its presence stimulates the growth of RGC axons. Expression of IGFBPL1 during development correlates with RGC axon growth capacity, and acute knockdown of IGFBPL1 with shRNA or IGFBPL1 knockout in vivo severely impaired RGC axonal growth. In contrast, administration of IGFBPL1 promoted axonal growth and regeneration in vitro and in vivo. IGFBPL1 activates the axon growth machinery through activating IGF-1-mediated calcium signaling to enable intracellular cascades required for axon elongation.

Conclusion: This finding reveals a novel regulatory element that is essential for the control of axonal growth cascades and an unexpected signaling loop in the pleiotropic functions of IGF-1. Considering that IGFBPL1 is widely expressed in the developing brain when neurons are extending axons, the results unveil a new therapeutic target for promoting optic nerve and CNS axon regeneration and repair.

Involvement of caspase-7 in optic nerve crush-induced retinal ganglion cell death

Iok-Hou Pang^{1,2}, Shreyasi Choudhury^{1,2}, Yang Liu^{1,2}, Abbot F. Clark^{2,3}

¹*Dept of Pharmaceutical Sciences,*

²*North Texas Eye Research Institute,*

³*Dept of Cell Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX*

Purpose: Optic nerve (ON) injury, which leads to apoptosis of retinal ganglion cells (RGCs), is associated with various ocular abnormalities, e.g., glaucoma, traumatic optic neuropathy, and compressive optic neuropathy. Caspases are important mediators of apoptosis. Some, such as caspase-3, are implicated in RGC death. However, the role of caspase-7, a functionally unique caspase, in ON injury has not been studied. This study was designed to evaluate the role of caspase-7 in ON injury-induced RGC death.

Methods: ON crush (ONC) was performed on C57BL/6J (WT) and Casp7^{-/-} mice. Western blots of retinal proteins were used to assess activation of caspase-7. Immunohistochemistry was performed to detect localization of caspase-7. RGC survival after ONC was determined by (1) counting RBPMS (RGC marker) labeled cells in flat-mounted retinas, (2) spectral-domain optical coherence tomography (SD-OCT), and (3) scotopic threshold response of ERG (STR).

Results: ON crush caused loss of RGCs, as determined by cell counts, retinal layer thickness, and STR. The injury activated caspase-7 in RGCs. Knockout of caspase-7 significantly protected against the injury-induced RGC loss. SD-OCT analysis revealed that the thickness of the ganglion cell complex layer in Casp7^{-/-} mice was significantly greater than WT mice at 28 d post ONC. STR amplitude was significantly higher (in Casp7^{-/-} mice at 28 d post injury).

Conclusions: Our findings indicate that caspase-7 plays a critical role in ONC-induced RGC death, and inhibition of caspase-7 activity may be a novel therapeutic strategy for glaucoma and other neurodegenerative diseases of the retina.

Deletion of adiponectin receptor 1 causes photoreceptor dysfunction and degeneration

Dennis S. Rice^{1,2} and Nicolas Bazan³

¹Lexicon Pharmaceuticals, 8800 Technology Forest Place, The Woodlands, TX 77381

²Novartis Institutes for Biomedical Research, Massachusetts Avenue, Cambridge, MA 02139 (current address)

³Neuroscience Center of Excellence, School of Medicine, Louisiana State University Health Sciences Center, 2020 Gravier Street, New Orleans, LA 70112

Purpose: Identification of molecular pathways involved in photoreceptor and retinal pigment epithelium physiology is a critical step in discovering therapies to treat blindness. A large-scale, functional genomics screen was conducted to identify genes that affect the outer retina in mice. Here, we report the discovery of adiponectin receptor 1 (AdipoR1) as a regulator of photoreceptor survival and function.

Methods: Adiponectin and AdipoR1 knockout mice were generated using retroviral gene trapping and homologous recombination. Postnatal and adult mice were evaluated using imaging, histology, electroretinography (ERG) and molecular profiling.

Results: Mice lacking AdipoR1 exhibited attenuated ERG responses at 4 weeks of age, concomitant with decreased 11-cis-retinal levels. At this age, TUNEL-positive cells were observed in the outer nuclear layer (ONL) in the knockout mice. LC-MS/MS-based lipidomic profiling revealed a substantial reduction in docosahexaenoic (DHA) levels and specific species of very long chain polyunsaturated fatty acids (VLC-PUFAs) in the retina. Retinal arachidonic acid (esterified and free) and systemic DHA levels were unchanged. Ex vivo explants obtained from AdipoR1 knockout mice, poorly incorporated deuterium labeled-DHA. Overexpression or silencing of AdipoR1 in human RPE cells resulted in enhanced or decreased DHA uptake, respectively. Knockout of adiponectin, a ligand of AdipoR1, was not associated with photoreceptor degeneration.

Conclusions: We have discovered a novel function for AdipoR1: it is required for the survival of photoreceptors in mice. AdipoR1 is a molecular switch that controls DHA retention and conservation, a key step to generate VLC-PUFAs in retinal photoreceptor cells.

Novel Strategy for RPE Protection: Glutaredoxin-Targeting Natural Products

Hongli Wu^{1,2}, Xiaobin Liu¹, Christy Xavier¹, Jamieson Jann¹, Dante Giordano¹, Benjamin Nguyen¹

¹ *Department of Pharmaceutical Sciences, University of North Texas System College of Pharmacy,*

² *North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, Texas, USA*

Purpose: Oxidative stress-induced retinal pigment epithelial (RPE) cell damage is known as an important factor in the pathogenesis of retinopathies, such as age-related macular degeneration (AMD). In our previous study, we identified glutaredoxin 1 (Grx1), a thiol-disulfide oxidoreductase, as a new cytoprotective enzyme in RPE cells. In this study, we searched for small molecule Grx1 inducers from natural products to protect RPE cells from oxidative damage.

Methods: Five natural antioxidant phenolics, including Salvianolic acid A (Sal A), Salvianolic acid B (Sal B), total salvianolic acid, curcumin, and epigallocatechin gallate (EGCG) were screened for their Grx1-inducing activities and cytoprotective effects in primary human RPE cells. Grx1 expression was examined by Western blot. Cell viability was evaluated with the WST8 assay. The level of protein glutathionylation (PSSG) was measured by using anti-PSSG antibody.

Results: Among all the tested compounds, Sal B was found to be the most potent Grx1 inducer, which upregulated Grx1 by ~3 fold at 50 μ M after 24 h. In both a time and dose-dependent manner, Sal B protected cells from H₂O₂-induced cell viability loss. Sal B also reduced annexin V positive cells, decreased Bax/Bcl-2 ratio, prevented caspase-3 cleavage, and inhibited ROS production. Additionally, H₂O₂-induced PSSG accumulation was markedly decreased by Sal B treatment. Moreover, knockdown of Grx1 by siRNA significantly reduced the cytoprotective effects of Sal B.

Conclusions: Sal B protects primary human RPE cells from oxidative stress-induced damage by upregulating Grx1. Naturally occurring Grx1 inducers may be used as new therapeutic strategies to treat oxidative stress-related retinopathies like AMD.

A novel neuroprotective small molecule for GDNF induction and photoreceptor rescue.

Petr Baranov¹, Hong Lin², Kathryn McCabe², David Gale², Shenshen Cai², Dwight Morrow², Phoebe Lei², Justin Liao², Michael Young¹

¹ *The Schepens Eye Research, Massachusetts Eye and Ear, an affiliate of Harvard Medical School;*

² *GlaxoSmithKline LLC*

Purpose. Retinal degenerative disorders, such as retinitis pigmentosa and age-related macular degeneration are characterized by irreversible loss of photoreceptors. Several growth factors, including glial-cell line derived neurotrophic factor (GDNF), have been shown to rescue retinal neurons in animal models of retinal disease. Previously we have observed GDNF induction in normal and diseased retina by amitriptyline. Our goal was to study the ability of a novel small molecule GSK812, a hit out of GSK GDNF induction phenotypic screen in ES-derived astrocytes and C6, to induce GDNF in vitro/in vivo and rescue photoreceptors in the rhodopsin knockout mouse model of retinal degeneration (rho -/-).

Methods. GDNF induction in vitro was assessed in rat C6 and human ARPE-19 cells. For time-course pharmacokinetic and GDNF induction studies in C57Bl/6 mice 2 ul of GSK812 slow release formulation (suspension, 60 mg/ml) were injected intravitreally. The same delivery approach was used in 4-week-old rho -/- mice to assess GDNF induction and photoreceptor rescue (14 weeks).

Results. The slow-release suspension provided sustainable GSK812 delivery with 28 ug of drug remaining in the eye 2 weeks after a single injection. GSK812 suspension injection in C57Bl/6 mice resulted in significant upregulation of GDNF on RNA (1.8 fold at day 7 and 14) and protein levels (2.8 and 3 fold at days 7 and 14, respectively). Importantly, GSK812 treatment resulted in ONL preservation in rho-/- mice with a 1.5-fold increase in photoreceptor number compared to control.

Conclusions. GSK812 is a potent neuroprotective compound that can induce GDNF in normal and diseased retina. This induction results in photoreceptor rescue in a mouse model of retinal degeneration.

Direct interaction of cochlin with mechanosensing channel TREK-1 in intraocular pressure regulation

Teresia A. Carreon¹, Carmen Piqueras¹, Xavier Gasull², Sanjoy K. Bhattacharya¹

¹*Bascom Palmer Eye Institute, University of Miami, Miami, FL, 33136.*

²*Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain, 08036.*

Purpose: To determine the direct interaction between the extracellular matrix protein cochlin and the mechanotransducing channel TREK-1 in intraocular pressure regulation

Methods: TREK-1 mRNA was silenced in the DBA/2J mouse model in an effort to determine its effect on intraocular pressure. A sodium fluorescein dye assay along with a gel expansion assay is implemented to mimic the effect of the cochlin/TREK-1 interaction on trabecular meshwork cell conformation. The interaction was further characterized by a co-immunoprecipitation and the utilization of a yeast – two hybrid system. Patch – clamp single channel recording was also performed in the presence or absence of cochlin in order to measure TREK-1 channel activity.

Results: The silencing of TREK-1 mRNA demonstrated the prevention of an increase in intraocular pressure (IOP) in the presence of cochlin. The presence of cochlin and TREK-1 together causes a spatial change in the cells of the trabecular meshwork as demonstrated by the increase of the fluorescein dye and the increase in length of the rat collagen in the gel expansion assay. A co-immunoprecipitation further demonstrated the increase of cochlin and TREK-1 specifically under shear stress and ionic stress conditions. The yeast-two hybrid system using a T7 promoter sequence demonstrated direct interaction between the bait (hCochlin) and TREK-1. Cochlin reduces TREK-1 channel current as demonstrated by single channel patch clamp recordings.

Conclusion: The results support the potential interaction occurring between cochlin and TREK-1 under stressful conditions, which in turn may result in extracellular matrix remodeling and an increase in IOP.

Evaluation of a Bioengineered Conventional Outflow Tract as a Model for High-throughput Glaucoma Drug Screening

Cula N. Dautriche^{1,2}, Dennis Szymanski¹, Matthew Kerr¹, Magnus Bergkvist¹, Yubing Xie¹, W.D. Stamer³, John Danias², Susan Sharfstein¹

¹ SUNY Polytechnic Institute, College of Nanoscale Science and Engineering, Albany NY 12203,

²Department of Ophthalmology, Downstate Medical Center, Brooklyn, NY 11203,

³Department of Ophthalmology, Duke University, Durham, NC 27710

Purpose: To engineer an in vitro biomimetic 3D conventional outflow tract as a model for high-throughput drug screening thus rapid development of trabecular meshwork-targeted therapies.

Methods: Using photolithography and microfabrication techniques, we have engineered a porous scaffold-hydrogel system for sequential seeding of human trabecular meshwork and Schlemm canal cells, mimicking the micro-architecture of the conventional outflow tract. We evaluated whether the co-culture system mimics the conventional outflow tract in terms of ultrastructure, morphology, physiology, and outflow functions via scanning electron microscopy (SEM) analysis, immunohistochemistry (IHC), perfusion studies to assess outflow facility and drug response studies

Results: SEM and IHC analysis revealed that the engineered tissue exhibits in vivo-like characteristics in terms of ultrastructure, key cell-specific marker expression (vascular endothelial cadherin, CD31, α -SMA, myocilin) and phagocytic activity. Treatment of the engineered tissue with the Rho kinase inhibitor, Y27632 increases the hydraulic conductivity from 3.22 to 5.55 $\mu\text{l}/\text{min}/\text{mm Hg}$ while treatment with the glauco-pathogenic agents such prednisolone acetate or TGF- β 2 decreases the outflow facility to 1.14 $\mu\text{l}/\text{min}/\text{mm Hg}$, each via mechanisms that mirror the response of the in vivo tissue such as inhibition of actin assembly, increase myocilin deposition or increase fibronectin expression, respectively, as confirmed by IHC analysis.

Conclusion: Our data suggest that our biomimetic conventional outflow tract exhibit outflow regulation as well as physiologic responses to well-established pharmacotherapies and may be used as a model for drug screening to help predict the performance and clinical success of glaucoma therapies.

Trabecular Meshwork Exosomes: Role in Cellular Uptake of ECM

W. Michael Dismuke¹ and W. Daniel Stamer¹

¹Duke University, Ophthalmology Dept, Durham, NC USA.

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 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. Fibronectin (Fn) conformation was monitored by detergent solubility. Collagen uptake was assayed by internalization of fluorescent collagen-1 coated or carboxylated microspheres.

Results: hTM cells release vesicles with size and protein composition characteristic of exosomes. These exosomes bind Fn and collagen-1 in a calcium-independent manner. LC-MS/MS revealed a known Fn-binding protein DPPIV, whose activity was detected in purified exosomes. Bound Fn exists in several conformations, including a deoxycholate and SDS insoluble polymer. Interestingly, a concurrent increase in hydrodynamic radius was measured in exosomes bound to Fn, possibly due to polymerized Fn enclosing the exosome in a “shell”. Finally, autologous and allogenic addition of excess hTM-derived exosomes to hTM cells, increased uptake of collagen-1-coated but not carboxylated microspheres, while Fn and collagen-IV secretion were unaffected.

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 this system can be manipulated to rescue aberrant ECM accumulation observed in glaucomatous TMs.

Pharmacological Responsive Bioengineered 3D Human Trabecular Meshwork

Karen Y. Torrejon¹, (Presenter), Justin Halman¹, Ellen Papke¹, John Danias², Magnus Bergkvist¹, Susan Sharfstein¹, Yubing Xie¹

¹*Colleges of Nanoscale Science and Engineering, SUNY Polytechnic Institute, Albany, NY, USA*

²*Department of Ophthalmology, SUNY Downstate Medical Center, Brooklyn, NY, USA*

Purpose: To establish and validate an in vitro 3D human trabecular meshwork (HTM) for high-throughput screening of biological agents that target HTM physiology.

Methods: We established a 3D HTM by culturing primary HTM cells on microfabricated, porous scaffolds. To test its feasibility for drug screening, we examined the pharmacological responsiveness to IOP-lowering agents (latruncunlin-B (Lat-B) and rho kinase inhibitor Y-27632) and glaucoma-inducing agents (prednisolone acetate (PA) and TGF- β 2). Samples were assayed for cellular expression and secretion of myocilin, ECM proteins and inflammatory cytokines. In parallel, perfusion studies were used to measure the outflow facility of 3D HTM in response to PA, TGF- β 2 and Y-27632. The perfused samples were also assessed by SEM, F-actin cytoskeleton organization and immunocytochemistry analysis of ECM deposition and electrical conductivity.

Results: The 3D HTM showed dose-dependent response to Lat-B and PA. PA and TGF- β 2 treatments increased expression/secretion of myocilin, ECM proteins and electrical conductivity compared to untreated control. The outflow facility exhibited 2-fold decrease after PA treatment and decreased to a greater extent after TGF- β 2 treatment. PA-perfused 3D HTM cells presented cross-linked actin networks and enlarged nuclei that are distinct from those treated without flow. In terms of cytokines, IL-1 α and TIMP-1 were upregulated while MMP3 and TNF α were downregulated after treatment. Exposure to Y-27632 decreased α -smooth muscle actin expression and increased outflow facility along with disrupted F-actin fibers.

Conclusions: Bioengineered 3D HTM responded to biological agents in a similar fashion to that seen ex vivo, confirming its applicability in high-throughput screening of agents affecting IOP.

An anti-angiogenic small molecule therapy for choroidal neovascularization

Rania S. Sulaiman^{1,2}, Halesha D. Basavarajappa^{2,3}, Bit Lee⁵, Xiang Fei⁵, Seung-Yong Seo⁵, Timothy W. Corson^{1,2,3,4}

¹*Department of Pharmacology and Toxicology*

²*Eugene and Marilyn Glick Eye Institute;*

³*Department of Biochemistry and Molecular Biology;*

⁴*Department of Ophthalmology; Indiana University School of Medicine, Indianapolis, Indiana, USA.* ⁵*College of Pharmacy, Gachon University, Incheon, South Korea.*

Purpose: Choroidal neovascularization is the main vision-threatening characteristic of wet age-related macular degeneration (AMD), a major cause of blindness in the elderly. Targeting the vascular endothelial growth factor (VEGF) system using biologics is the standard treatment for this disease. However, such biologics are associated with ocular and systemic side effects, and some patients are refractory. Therefore, there is a need for small molecule treatments for wet AMD. We previously synthesized an anti-angiogenic homoisoflavanone, cremastranone, with anti-angiogenic effects on human retinal endothelial cells (HRECs). Here, we present a synthetic analog of cremastranone, SH-11037, with remarkable potency and selectivity in vitro, as well as anti-angiogenic efficacy in vivo without ocular toxicity.

Methods: The anti-proliferative activity of SH-11037 was tested on several cell lines using the alamarBlue proliferation assay. The in vitro anti-angiogenic effects of SH-11037 were studied using EdU incorporation assays, migration, and tube formation assays, and immunofluorescence. The laser-induced choroidal neovascularization (L-CNV) mouse model was used to test the anti-angiogenic activity of SH-11037 in vivo. Toxicity of intravitreally injected SH-11037 was examined by histology and immunohistochemistry.

Results: SH-11037 dose-dependently inhibited HREC DNA synthesis, migration, and tube formation at nanomolar concentrations without causing apoptosis or cytotoxicity in other cell types. SH-11037 significantly suppressed angiogenesis in the L-CNV model. Moreover, short- and long-term toxicity studies established the absence of ocular toxic effects of SH-11037 after intravitreal injections.

Conclusions: These data demonstrate the strong anti-angiogenic potential of SH-11037, which is an exciting lead for the development of a small molecule treatment for wet AMD.

Identification of NA3 glycan as a potential candidate for atrophic AMD by promoting the development of photoreceptor outer segment assembly via receptor ligation in Muller cells

Sumana R. Chintalapudi¹, Liyuan Li¹, Huiling Li^{1,4}, Mallika Palamoor¹, Xiaofei Wang¹, Vanessa Morales Tirado¹, Monica M. Jablonski^{1,3}

¹Ophthalmology, Hamilton Eye Institute, University of Tennessee Health Science Center, Memphis, TN, United States.

²Microbiology, Immunology and Biochemistry, University of Tennessee Health Science Center, Memphis, TN, United States.

³Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN, United States.

⁴Department of Ophthalmology, The Second Xiangya Hospital, Central South University, Changsha, China. 410011.

Purpose: Atrophic age-related macular degeneration is the most common type of AMD. It accounts for approximately 90% of all cases, yet there is no available therapy. Atrophic AMD is characterized by damage to the macula, affecting the support of photoreceptors and ultimately, visual function. Our lab previously showed lactose and structurally related glycans support photoreceptor outer segment (OS) structure in the absence of retinal pigment epithelium (RPE). The present study identifies the responsible glycan receptor and investigates its potential therapeutic use.

Methods: We evaluated the ultrastructure of OS in *X. laevis* embryos after RPE removal. Eyes were cultured under different glycan conditions to evaluate function, effective dose and treatment. NA3 binding affinity against competing glycans was assessed by immunohistochemistry analysis and flow cytometry in primary murine cells. Human retinae sections were also evaluated for NA3 binding affinity by IHC. The *in vivo* binding pattern was analyzed in NZW rabbits by autoradiography.

Results: We have demonstrated for the first time that NA3 supports photoreceptor OS assembly with high affinity via receptor-mediated response. The glycan is safe and has no deleterious effect on either retinal structure or protein expression patterns. FACS and immunostaining studies reveal that NA3 binds to Müller cells. Results were consistent among the three species (*Xenopus*, mice and human). *In vivo* work in NZW rabbits supports these results, confirming that NA3 binds to Müller cells.

Conclusions: We identified NA3 amongst plausible glycan candidates as a potential novel therapy for atrophic AMD by promoting the development of photoreceptor OS.

Nerve fiber density in the corneal subbasal plexus, its role in the follow-up of diabetic neuropathy

Rudolf F. Guthoff¹, Oliver Stachs²

¹*Institute for Biomedical Engineering, Rostock University Medical Center, Friedrich-Barnewitz Strasse, D-18119 Rostock, Germany*

²*Department of Ophthalmology, Rostock University Medical Center, Doberaner Strasse 140, D-18057 Rostock, Germany*

Purpose: The small fiber neuropathy is a frequent and poorly understood complication in diabetic patients. Clinical tests, assessing neuropathy predominantly focus on large fiber deficits, yet the earliest alterations occur in the small unmyelinated nerve fibers. The diagnostic armamentarium for small fibers evaluation in diabetes is mainly based on semiquantitative sensory skin testing including tuning fork, and skin biopsy to investigate small caliber sensory nerves histologically.

Methods: In recent years, in vivo confocal microscopy has been increasingly used to image the structure of the subbasal nerve plexus in health and disease. The non-invasive nature of confocal microscopy has the advantage of enabling examination of human cornea in its physiological state over time.

Results: In diabetes, changes in subbasal nerve morphology could be demonstrated by several groups worldwide. Our and other groups have demonstrated clinically and experimentally, that the in vivo confocal imaging modality is able to collect and accurately quantify changes in subbasal nerve plexus in diabetes, even in early stages of disease. The alterations of subbasal nerves encompass decrease of nerve fibre length, density and number of branches, and increased tortuosity coefficient.

Conclusions: Despite some instrumentation and quantification approaches-based discrepancies in absolute values reported by different groups, most reported finding on subbasal nerves parameters in diabetes are in good agreement, showing overall a decrease in nerve density and branching. These parameters have become a surrogate marker for early detection of diabetic neuropathy.

Utility of *In Vivo* Confocal Microscopy-Based Imaging Endpoints for the Assessment of Ocular Surface Inflammation

Pedram Hamrah, MD, FACS

Purpose: Recent studies have shown the role of immune changes in the pathogenesis of dry eye disease (DED) and a sensitive test is needed to quantify the immune response. The purpose of this study is to evaluate the utility of *in vivo* confocal microscopy changes in corneal and conjunctival inflammation to therapeutic intervention.

Methods: A phase IV, randomized, vehicle-controlled, double-masked, single-center clinical trial was conducted with 54 subjects clinically diagnosed with EDE that received either steroid alone (Loteprednol Etabonate 0.5%, LE, n=17), steroid with antibiotic (Loteprednol Etabonate 0.5% + Tobramycin, LE/T, n=17), or artificial tears alone (AT, n=20) for 4 weeks with quantification of central corneal DC densities on IVCN pre- and post-treatment. Sixty-two healthy reference controls were also included for comparison.

Results: At baseline, subjects with DED had significantly increased corneal dendritic cell (DC) ($P < 0.001$), conjunctival epithelial immune cell (EIC) ($P < 0.01$) and stromal immune cell (SIC) densities ($P = 0.01$), with no differences between the treatment groups at baseline ($P = 0.59$). Following treatment, DC, EIC, and SIC densities reduced in both the steroid-containing treatment groups, LE/T and LE ($P < 0.01$), but not AT ($P = 0.44$) demonstrating specificity of response. DC density correlated with corneal fluorescein staining (CFS) ($R = 0.48$, $P < 0.0001$), OSDI scores ($R = 0.37$, $P < 0.0001$), and TBUT ($R = -0.25$, $P < 0.01$). CFS and OSDI scores improved by 24% ($P = 0.03$) and 11% ($P = 0.06$) in the LE-treated group, and by 21% ($P = 0.06$) and 15% ($P = 0.09$) in the AT-treated group, respectively.

Conclusions: Corneal DCs and EIC as indicators of tissue inflammation are increased in ocular surface disease and their quantification *in vivo* allows for detection of changes in tissue inflammation following anti-inflammatory therapy in dry eye, providing responsive endpoints. Given the significant correlation of corneal DC density to both symptoms and clinical signs, this *in vivo* imaging parameter may serve as a surrogate biomarker of therapeutic efficacy in clinical trials.

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Commercial disclosure: Patent Application Pending

In vivo confocal microscopy of the ocular surface in dry eye: over the cornea

Edoardo Villani

Purpose: To explore potentials of in vivo confocal microscopy (IVCM) to study the ocular surface morpho-functional unit and to provide valuable biomarkers for diagnosis and follow-up

Methods: IVCM was applied to several components of the ocular surface in healthy subjects, in different types of dry eye patients, and in other ocular surface diseases.

Results: IVCM provided important information on central and peripheral cornea, bulbar and tarsal conjunctiva, eyelid margin, and meibomian glands. Some confocal parameters showed good repeatability and reproducibility and were able to discriminate different pathogenic processes with similar clinical features and to detect response to treatments.

Conclusion: IVCM of non-corneal components of the ocular surface is a new and interesting application of in vivo microscopy to ocular diseases. Several challenges still remain, but this approach promises to give an important contribution to the personalization of diagnosis and disease management.

Towards development of improved anti-angiogenic therapies: insights from in vivo confocal microscopy in the murine cornea

Neil Lagali, Pierfrancesco Mirabelli, Anthonny Mukwaya, Maria Xeroudaki, and Beatrice Peebo.

Department of Ophthalmology, Institute for Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

Purpose: To assess the efficacy of pharmacotherapy for blood vessel regression in murine models of corneal angiogenesis, using intravital in vivo imaging.

Methods: Wistar rats receive single or several intrastromal corneal sutures to induce a controlled inflammatory angiogenic response. After suturing, topical or subconjunctival anti-angiogenic therapy is given. Starting from hours after suture placement to 4-5d, rat corneas are examined in vivo at the single-cell and single-vessel level by laser-scanning corneal confocal microscopy. Sutures are removed, and corneas are re-examined in vivo while vessels regress.

Results: By in vivo confocal microscopy, the earliest stages of inflammatory cell infiltration and accumulation can be visualized and quantified. Later accumulation of mature macrophages at the suture site can also be unambiguously identified and quantified. Dilation of limbal vessels can also be easily quantified, and different forms of early blood vessel growth can be distinguished, such as sprouting and looping angiogenesis. Moreover, in regression the anti-angiogenic role of macrophages, vascular pruning, blood flow inhibition, and resistive mechanisms to anti-angiogenesis can be identified and followed in a time-dependent manner in the same corneas.

Conclusions: In vivo confocal microscopy of the rat cornea is a valuable tool for longitudinal, cellular-level investigations of inflammation, blood vessel growth and regression in response to pharmacotherapy.

“Imaging models for assessing angiogenesis and evaluating anti-angiogenic therapies”

Beatrice B Peebo, Pierfrancesco Mirabelli, Anthonny Mukwaya, Maria Xeroudaki, and Neil Lagali.

Department of Ophthalmology, Institute for Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

Purpose: To assess angiogenesis, blood vessel maturation and the effect of anti-angiogenic therapies using intra-vital imaging in two neovascularization models.

Methods: Corneal neovascularization model: Wistar rats received an intrastromal corneal suture to induce a controlled inflammatory angiogenic response. Rats were subsequently treated with dexamethasone, rat specific anti-VEGF, or goat IgG (control), topically. In vivo confocal microscopy of the cornea was performed up to 7 d to investigate morphology at the cellular and tissue-level. In vivo photographic vessel analysis were also performed. RT-PCR for angiogenic and inflammatory cytokines were additionally done.

Hypoxia-induced retinopathy in adult zebrafish: Adult fli1:EGFP zebrafish are placed in hypoxic water for 3-10 d and retinal neovascularization is analyzed ex-vivo using confocal fluorescence microscopy.

Results: Blood vessel sprouting, growth and maturation was possible to assess in a time-lapse manner in both models. Anti-angiogenic treatments influence early, pre-angiogenic activity such as limbal vessel dilation, inflammatory cell infiltration and sprouting. The differential effects of steroids and anti-VEGF treatment in suppressing neovascular growth could not be attributed to differential inhibition of several major angiogenic and inflammatory factors in the early pre-sprouting phase.

Conclusions: The rat corneal neovascularization and zebrafish hypoxia-induced retinopathy models are suitable for assessing in vivo blood vessel formation, maturation and, in addition, for evaluating the effect of anti-angiogenic therapies.

Immune Responses at the Ocular Surface

Daniel R. Saban, PhD^{1,2}

Departments of Ophthalmology¹ and Immunology², Duke University School of Medicine, Durham, NC, USA

Immune-mediated diseases of the ocular surface are relatively broad in their respective etiologies, which can involve infection, autoimmunity, or allergy. Despite this range, immune responses often converge upstream at the level of the dendritic cell (DC)—a highly specialized group of antigen presenting cells required in the activation of naïve T cells. Our lab has established a novel mouse model of allergic eye disease (AED), which leads to severe clinical manifestations, sustained ocular inflammation, and eosinophilic infiltration at levels seen in patients with atopic keratoconjunctivitis. Use of the AED model has led to identification of classical CD11b⁺ DCs as the key subset responsible for activating allergen reactive T cells. In addition, the AED model has uncovered the importance of CCR7 as the master chemokine receptor in homing of ocular surface DCs to the regional lymph nodes. Additionally, CCR7 has recently been shown to contribute to activation of Th17 cells in the mouse model of dry eye disease. Likewise, similar to the dry eye disease model, AED involves corneal lymphangiogenesis—potentially suggesting a role for corneal DCs in allergic immune responses. Thus, efforts to progress the current understanding of DC biology holds tremendous promise for advancement of novel and effective medicines in immune mediated diseases of the ocular surface.

Ocular Autoimmunity – a collusion of development and environment

Rachel R Caspi, *Laboratory of Immunology, NEI, NIH*

Non-infectious uveitis of a putative autoimmune nature is responsible for up to 15% of blindness. Autoimmune uveitis is believed to be driven by retina-specific T cells that were activated in the periphery and have acquired the ability to actively cross the blood-retinal barrier into the eye, but the reason for their presence and where they become activated, are unknown. This presentation will explore these two issues, and present evidence that retina-specific T cells that escaped negative selection in the thymus during development and have not undergone peripheral tolerance due to lack of retinal antigens in the periphery, persist in the body in an ignorant, but not tolerant, state. They may become activated in the gut by crossreactive antigens derived from commensal microbiota, which are delivered in the context of a built-in bacterial adjuvant, whereupon they differentiate into effector T cells capable of reaching the eye and eliciting autoimmune inflammation.

T Cells in Ocular Dysfunction: Lessons From a Vitiligo Prone Novel Transgenic Mouse

Shikhar Mehrotra¹ and Shahid Husain²,

¹*Department of Surgery, Hollings Cancer Center, Medical University of South Carolina, Charleston, SC 29425*

²*Hewitt Laboratory of the Ola B. Williams Glaucoma Center, Department of Ophthalmology, Storm Eye Institute, Medical University of South Carolina, Charleston, SC 29425.*

Purpose: Identification of tumor associated antigen have led to the usage of high affinity T cell receptors for engineering patients T cells with specificity to recognize tumors. Using the human tyrosinase epitope reactive, CD8 independent, high affinity TCR isolated from tumor infiltrating lymphocytes of a metastatic melanoma patient, we developed a novel TCR transgenic mouse (named h3T for human TIL1383I Tyrosinase TCR) on HLA-A2 background. This double transgenic mice h3T-A2 mouse developed spontaneous depigmenting Vitiligo and had visual defects that progressed with age. We further evaluated if IFN γ secreted by ocular infiltrating T cell is responsible for neurodegeneration/retina dysfunction and retinal ganglion cell (RGC) death in h3T-A2 mice.

Methods and Result: While CD3 and IFN γ levels were significantly increased in h3T-A2 mice, there was a concomitant decrease in RGC numbers (37%, $p=0.0001$), and function (42%, $p=0.03$) as measured by pattern electroretinograms (ERGs) when compared with wild-type mice. Both CD3 and IFN γ immunostaining was increased in nerve fiber (NF) and RGC layers of h3T-A2 mice. Similarly, infiltrating T cells was also confirmed in a chronic glaucoma rat model by injecting 50 μ L of 2.0 M hypertonic saline into limbal veins, at the 42nd day, post glaucomatous injury.

Conclusion: h3T-A2 transgenic mice exhibit visual defects that are mostly associated with the inner retinal layers and RGC function due to increased T cells infiltration and IFN γ production. Overall, this novel h3T-A2 transgenic mouse model provides an opportunity to understand RGC pathology and could be highly useful for testing neuroprotective strategies to rescue RGCs.

Immunology of retinal degenerative disease

Heping Xu, *Centre for Experimental Medicine, School of Medicine, Biomedical Sciences and Dentistry, Queen's University Belfast, UK*

Purpose: To review retinal immune response to various endogenous insults and the contribution of dysregulated immune response to retinal degenerative disease.

Method: Published and unpublished data from personal study in in vivo models and literature review.

Results: The retina is considered as an immune privileged tissue, yet many inflammatory diseases occur. Retina has its own innate immune system, consisting of microglia, perivascular macrophages, a small number of dendritic cells and the complement system that protects the retina from exogenous and endogenous insults. Dysregulation or malfunction of the innate immune response may result in excessive production of inflammatory mediators that may contribute to retinal pathology. The presentation will discuss how innate immune response is controlled in the retina, and the potential contribution of uncontrolled or dysregulated innate immune activation to sight-threatening retinal degenerative diseases such as age-related macular degeneration and diabetic retinopathy.

Conclusions: Innate immune activation is important to maintain retinal homeostasis. Uncontrolled or dysregulated innate immune activation may contribute to the pathogenesis of various retinal degenerative diseases.

RNA Based Gene Therapy for Autosomal Dominant Retinitis Pigmentosa

Alfred S. Lewin

Purpose: We have been developing adeno-associated virus (AAV) vectors to deliver small RNA molecules for the treatment of autosomal dominant retinitis pigmentosa (adRP) associated mutations in RHO, the gene for rhodopsin. Allele-specific hammerhead and hairpin ribozymes blocked expression of mutant rhodopsin and led to long-term retinal preservation in a rat model of adRP. However, the allelic heterogeneity of adRP causing mutations in RHO make this approach impractical, so that we are taking an allele-independent approach.

Methods: AAV vectors were developed to deliver short interfering RNAs (siRNA) or ribozymes to deplete endogenous rhodopsin mRNA (mutant and wild-type) together with a ribozyme- or siRNA-resistant RHO cDNA to restore expression of normal rhodopsin protein. The siRNA genes are expressed either as short hairpin RNAs (shRNA) under the control of an RNA polymerase III promoter or as artificial microRNAs (a-miR) under the control of the RHO proximal promoter.

Results: Subretinal injection of a single AAV vector expressing an siRNA and a replacement gene lead to preservation of retinal structure, based on SD-OCT and histology, and function, based on full field ERG, for up to nine months of age in P23H rhodopsin transgenic mice.

Conclusions: Single vectors delivering both an RNA agent and a replacement gene can be an effective treatment for autosomal dominant retinitis pigmentosa associated with RHO mutations. This approach is now being evaluated in large animal models of this disease.

microRNAs in Diabetic Retinopathy and its Treatment

Shunbin Xu^{1,2}, Beatrix Kovacs^{3,4}, Colleen Cowan^{3,4,5}, Chithra K. Muraleedharan^{1,2}, James J. O'Donnell III⁵, Hazel Lum⁵, Stephen Lumayag^{3,4}, Sanja Turturro^{3,4,5}, Pei Zhuang^{1,2}

¹*Department of Ophthalmology, Kresge Eye Institute*

²*Department of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, Michigan*

³*Department of Ophthalmology*

⁴*Neurological Sciences and* ⁵*Pharmacology, Rush University Medical Center, Chicago, Illinois*

Purpose: The purpose of our study is to identify miRNAs involved in diabetic retinopathy (DR) and test the potential of candidate miRNAs as therapeutic targets for the treatment of DR.

Methods: miRNA expression profiling was performed in retinal endothelial cells (RECs) and retina of streptozotocin (STZ) diabetic rats, as well as in the retina of diabetic patients and normal control subjects. In vitro overexpression and knockdown experiments were conducted in human RECs. Lentivirus expressing pre-miR-146 and miR-146 inhibitor were produced and injected intravitreally and intravenously into STZ-induced diabetic rats to evaluate the potential of miR-146 as a therapeutic target for treatment of DR.

Results: 1) We identified a series of miRNA signatures reflecting ongoing pathological changes in RECs and the retina in diabetic rats. These signatures include a) NF- κ B responsive miRNAs; b) VEGF responsive miRNAs, and c) apoptosis and cell senescence related miRNAs. Some of these signature miRNAs showed similar changes in the retina of diabetic patients; 2) We demonstrated that miR-146 has negative feedback regulations on both IL-1R/TLR-mediated and G-protein-coupled receptor mediated NF- κ B activation pathways by targeting key adaptor molecules in these pathways in RECs; 3) In vivo delivery of miR-146 and miR-146-inhibitor by lentiviral infection resulted in changes of expression levels of miR-146 and several key downstream target genes in the eye, liver and several other organs.

Conclusions: miRNAs are involved in multiple pathological pathways of DR. miRNAs are novel therapeutic targets for the treatment of DR and other diabetic complications.

Targeted Nanocarriers for Therapy of Retinal Vascular Disease

Ashwath Jayagopal^{1,2}

¹ *Vanderbilt Eye Institute, Vanderbilt University Medical Center*

² *Molecular Physiology and Biophysics, Vanderbilt University School of Medicine*

Purpose: Cell adhesion molecules (CAMs), particularly ICAM-1 and VCAM-1, are markers of inflammation expressed on retinal endothelial cell surfaces in a broad spectrum of ocular vascular diseases, including retinal neovascularization. We have developed targeted polymeric nanocarriers targeted against CAMs which can bear imaging or therapeutic payloads and deliver them to the cytoplasm of dysfunctional endothelial cells. The goal of this study was to demonstrate the utility of CAM targeted nanocarriers for intracellular delivery of antiangiogenic siRNA or anti-miRNA in two mouse models of retinal neovascularization.

Methods: CAM targeted nanocarriers bearing VEGFR2 and other siRNAs were characterized to determine optimal size, surface charge, and encapsulation efficiencies. Cytotoxicity, delivery efficiency, and functional knockdown of several molecular targets were determined in retinal microvascular endothelial cells. Biodistribution and efficacy of nanocarriers in mouse models of laser induced choroidal neovascularization and oxygen-induced retinopathy were analyzed.

Results: CAM targeted nanocarriers were capable of specific targeting of the CAMs ICAM-1 and VCAM-1 on inflamed retinal endothelial cells *in vitro* and *in vivo*. Specific targeting of inflamed retinal endothelium was observed in both animal models of vascular disease, using CAMs on neovessel endothelial cells as a portal for delivery of therapies. Knockdown of several molecular targets via siRNAs was achieved *in vitro* and *in vivo*. Nanocarriers loaded with siRNAs or anti-miRNAs were capable of inhibiting neovascularization in both animal models.

Conclusions: CAM targeted nanocarriers are a promising framework for the delivery of diverse imaging and therapeutic payloads to the cytoplasm of diseased retinal endothelial cells *in vivo*.

Noncoding RNAs in ocular angiogenesis

Susheng Wang, *Tulane University*

Purpose: Research in my lab is focused on elucidating the functional mechanism and exploring therapeutic potential of noncoding RNAs including microRNAs (miRNA or miR) and long noncoding RNAs (lncRNA) in retinal vascular diseases. miRNAs have been emerged as pivotal modulators of vascular development and disease. We have shown that miR-23 and miR-27 in miR-23~27~24 family are required for proper angiogenesis and neovascularization in a laser-induced choroidal neovascularization (CNV) model. The goal of the project is to define the function of miR-24 in ocular angiogenesis.

Method: Laser injury AMD model, in vivo miRNA mimic delivery methods, as well as in vitro systems using endothelial cells (EC) were used to dissect the function of miR-24 in angiogenesis and CNV.

Results: We show that miR-24 regulates actin dynamics in ECs through targeting multiple members downstream of Rho signaling, including Pak4, Limk2 and Diaph1 proteins. Subretinal delivery of miR-24 mimics represses laser-induced CNV in vivo. Mechanistically, knockdown of miR-24 target protein LIMK2 or PAK4 inhibits stress fiber formation and tube formation in vitro, mimicking miR-24 overexpression phenotype in angiogenesis.

Conclusion: These findings demonstrate that miR-24 represses angiogenesis by simultaneously regulating multiple components in the actin cytoskeleton pathways, suggesting distinct function of miR-23~27~24 family members in angiogenesis. Manipulation of actin cytoskeleton pathways by miR-24 may represent an attractive therapeutic solution for retinal vascular diseases. The functional role of several other key miRNAs in retinal vascular development and disease will also be discussed.

AON intravitreal injections to manipulate splicing in retinal cells

Xavier Gérard, Isabelle Perrault, Arnold Munnich, Josseline Kaplan, Jean-Michel Rozet
Institut Imagine, *Ophthalmic Genetics, Paris, France*

Purpose: Leber congenital amaurosis (LCA) is the leading cause of hereditary blindness in children. CEP290 encodes a ciliary protein important to photoreceptor connecting cilium assembly and function. The CEP290 intronic c.2991+1655A>G change is the most common LCA-causing mutation (10%). It introduces a cryptic exon in the mRNA encoding a premature termination codon. Recently, we made the proof-of-concept of antisense oligonucleotides (AON)-mediated exon skipping to correct the splicing in patient fibroblasts which recovered ability to ciliate. The purpose of this study was to make the proof-of-concept of exon-skipping in vivo using intravitreal injections of AONs.

Methods: AONs were designed to skip mouse Cep290 exon 36. Variable concentrations of 6-FAM-AONs were injected into the vitreous of C57BL/6J mice. Retinal sections and mRNA were prepared during 30 days post-injections (dpi) to follow i) the distribution of oligonucleotides across cellular layers and ii) exon skipping efficiency.

Results: The most efficient AONs identified by in vitro analyses were injected in the vitreous of animals. Excellent correlation between the efficiency of exon skipping and AON injected dose has been demonstrated. Histological analyses revealed a wide distribution of AON in all retinal cell layers at least until 30 dpi. A linear amount decrease of mRNA lacking exon 36 was measured but still detectable at 30 dpi.

Conclusion: Here we report that single intravitreal injection of AON allows efficient and persistent exon skipping in retinal cells. Hence, this strategy may be regarded as an attractive alternative to gene replacement therapy for 10 % of patients affected with LCA.

The Suprachoroidal Space Accessed by Microneedle Injection

Bryce Chiang¹, Yoo Chun Kim², Samirkumar R. Patel^{2,3}, Henry F. Edelhauser^{3,4}, and Mark R. Prausnitz^{1,2}

¹*Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA*

²*School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA*

³*Cleaside Biomedical Inc., Alpharetta, GA; 4Emory Eye Center, Emory University, Atlanta, GA*

Traditional ophthalmic drug delivery methods, namely topical eye drops and intravitreal injections, do not offer targeted delivery to the posterior segment for the treatment of retinal diseases. Eye drops have low bioavailability past the anterior segment, and intravitreal injections rely on passive diffusion, which will result in much of the drug not diffusing towards the diseased tissue. The delivery of therapeutics into the suprachoroidal space (SCS) offers an exciting new ophthalmic drug delivery technique that is both targeted to specific tissues within the posterior segment and minimally invasive. Since the SCS is bordered by the choroid and sclera, deposition of drugs into this space results in highly localized delivery to the chorioretinal complex. A hollow microneedle with a length matched to the thickness of the sclera can be used to access the SCS in a procedure akin to an intravitreal injection. We will review the discovery of this drug delivery method, and describe current efforts to both better characterize the SCS and develop strategies for improved targeting. On-going studies are bringing suprachoroidal delivery using microneedles into the clinic.

Pharmacokinetic/Pharmacodynamic Considerations with Ocular Drug Delivery Implants

Michael R. Robinson¹ and Susan S. Lee¹

¹ Allergan, Inc., Irvine, CA

Purpose: Development of sustained-release drug delivery implants for treating eye diseases involving the posterior segment and can reduce the patient burden of requiring frequent intraocular injections to maintain therapeutic drug levels. Corticosteroids were the first drug class to be developed as drug-eluting ocular implants for treating a number of ocular diseases. Herein, we describe the pharmacokinetic, pharmacodynamics and toxicologic considerations when developing a corticosteroid-eluting implant for the eye.

Methods: Published literature review using PubMed on commercially available corticosteroid-eluting ocular implants.

Results: Three commercially available corticosteroid-eluting implants are available in the US and are indicated for the treatment of non-infectious uveitis affecting the posterior segment (Retisert® [fluocinolone acetonide], OZURDEX® [dexamethasone]), macular edema following retinal vein occlusion (OZURDEX®), diabetic macular edema (OZURDEX®), diabetic macular edema in patients who have been previously treated with a course of corticosteroids and did not have a clinically significant rise in intraocular pressure (Iluvien® [fluocinolone acetonide]). The pharmacokinetic drug profile of OZURDEX® demonstrates a very high drug level initially ('Pulse' dose) over 2 to 3 months followed by a longer, low-dose period out to at least 6 months. In contrast, the Retisert® and Iluvien® implants release low levels of corticosteroid in the eye for up to 3 years. The efficacy and risk differential between implants is influenced by the drug release profile and will be discussed.

Conclusions: Ocular drug delivery implants are a valuable treatment option for treating chronic eye diseases that involve the posterior segment. Pharmacokinetic and pharmacodynamics considerations are important during the development process to optimize patient visual outcomes.

Photokinetic Drug Delivery: Light-enhanced Permeation In An In Vitro Model Eye

Steven A. Giannos, Bernard F. Godley, Edward R. Kraft

University of Texas Medical Branch, Department of Ophthalmology and Visual Sciences, Galveston, TX 77555

Purpose: To investigate light enhanced molecular movement as a potential technology for drug delivery. To do this, an in-vitro model eye representing similar concentration gradient conditions and compositions found in the eye was developed.

Methods: The model-eye unit was fabricated by inserting a cross-linked type I collagen membrane in a spectrophotometer cuvette with 1% hyaluronic acid as the drug recipient medium. Photokinetic delivery was studied by illuminating 1mg/ml methotrexate (MTX) placed in the drug donor compartment on top of the membrane with non-coherent 450 nm light at 8.2 mW from an LED source pulsed at 25 cycles per second, placed in contact with the solution. A modified UV-visual spectrophotometer was employed to rapidly determine the concentration of MTX, at progressive 1mm distances away from the membrane, within the viscous recipient medium of the model eye after one hour.

Results: A defined, progressive concentration gradient was observed within the non-agitated drug recipient media, diminishing with greater distances from the membrane. Transport of MTX through the membrane was significantly enhanced (ranging from 2 to 3 times, $P < 0.05$ to $P \leq 0.001$) by photokinetic methods compared to control conditions by determining drug concentrations at four defined distances from the membrane. According to scanning electron microscopy (SEM) images, no structural damage or shunts were created on the surface of cross-linked gelatin membrane.

Conclusion: The application of pulsed non-coherent visible light significantly enhances the permeation of MTX through a cross-linked collagen membrane and hyaluronic acid receptor medium without causing structural damage to the membrane.

Nanoparticles in Ocular Drug Delivery

Kim Brazzell, PhD, Kala Pharmaceuticals

*University of Texas Medical Branch, Department of Ophthalmology and Visual Sciences,
Galveston, TX 77555*

Nanotechnology involves the manipulation of matter at the atomic and molecular scale. This rapidly growing area has significantly impacted various fields, including medicine. It has enabled enhanced medical imaging, more sensitive diagnostics, targeted drug delivery, non-viral gene delivery and tissue regeneration. Nanotechnology has made its way into pharmaceuticals to significantly improve the delivery and efficacy of drugs in a number of therapeutic areas, with several nanoparticle-based products currently on the market. In recent years there has been increasing evidence that nanotechnology can help overcome many of the ocular delivery barriers, allowing enhanced penetration and duration of delivery to various ocular tissues, including the back of the eye, and delivery of genes to ocular tissues without the need for viral vectors. A number of examples of enhanced drug delivery via nanoparticles will be presented as well as a review of some of the companies now working to apply nanotechnology to the better treatment of ocular disease. The utilization of mucus penetrating nanoparticle technology for enhancing delivery of drugs to both the front and back of the eye will also be discussed.

The Monthly Eye Drop: Preclinical testing of long-term, hydrogel/microsphere eye drops for glaucoma

Morgan V. Fedorchak^{1,2,3,6}, Ian Conner^{1,2}, Joel Schuman^{1,2,4,6}, Steven R. Little^{3,4,5,6}

¹University of Pittsburgh School of Medicine Department of Ophthalmology

²The Louis J. Fox Center for Vision Restoration, University of Pittsburgh Department of

³Chemical Engineering ⁴Bioengineering, and ⁵Immunology, and ⁶The McGowan Institute for Regenerative Medicine

Purpose: IOP reduction in patients with glaucoma is typically accomplished through the administration of medicated eye drops several times daily, with compliance rates as low as 30%. The purpose of this study was to develop and test a novel formulation that provides one month of glaucoma medication from a topical depot. We hypothesize that this treatment will address the issues of compliance and poor bioavailability while avoiding more invasive techniques.

Methods: Poly(lactic-co-glycolic) acid (PLGA) microparticles containing brimonidine tartrate (BT) were fabricated according to standard methods and mixed into a poly-(N-isopropylacrylamide) (pNIPAAm)-based hydrogel. The properties of the materials were characterized, including cytotoxicity and in vitro release of BT. For in vivo studies, a single drop was administered to the inferior fornix of New Zealand white rabbits and compared to twice daily BT drops.

Results: BT-loaded microparticles release sufficient drug over 28 days in vitro, which is unaffected by the presence of the gel. Cytotoxicity testing shows no significant effect on cell viability. The gel/microparticle drops are easily administered and form a non-degradable, opaque gel upon reaching body temperature. IOP reduction for a single administration of the hydrogel drops was comparable to twice-daily BT drops, with significantly lower systemic uptake. There was no evidence of irritation or inflammation.

Conclusions: The BT-loaded microparticles presented in this study are capable of releasing drug for four weeks. IOP reduction is achieved using 56 times fewer doses than the current clinical standard, representing a vast improvement in bioavailability and dosing frequency over current topical methods.

Perfusion Pressure: Continuous Telemetry Measurement of Blood Pressure and Bilateral IOP in Nonhuman Primates

J. Crawford Downs, *Department of Ophthalmology, University of Alabama at Birmingham School of Medicine, Birmingham, AL, USA*

Purpose: To test the hypothesis that bilateral ocular perfusion pressure (OPP) differs between nonhuman primates (NHP) as characterized via continuous radio telemetry of bilateral IOP and aortic blood pressure (BP).

Methods: We used an implantable wireless IOP/OPP telemetry system to record continuous bilateral IOP and aortic blood pressure (BP) at 500 Hz, 24 hours/day for 60-70 days in four young adult male rhesus macaques aged 3-6 years old. Bilateral IOP measurements were taken in 3 NHPs and unilaterally in 1 NHP. The IOP transducers were calibrated directly via anterior chamber manometry, and OPP was calculated 500 times per second as: central retinal artery (CRA) BP – IOP. The CRA systolic and diastolic BPs were calibrated directly to the telemetric aortic BP data via ophthalmodynamometry, by visualizing the IOPs at which the CRA begins to flutter (diastolic) and fully collapse (systolic). OPP data were corrected for signal drift, and averaged hourly.

Results: OPP was consistent between contralateral eyes but varied widely between animals, ranging from daily averages of ~17 mmHg to ~62 mmHg. OPP was lowest during the night, increased upon waking and was highest in late afternoon in all four NHPs. Nycthemeral fluctuations in mean OPP also varied widely between animals.

Conclusions: Average OPP varies widely between NHPs, is consistent between contralateral eyes within NHPs, is highest in afternoon and evening, and is lowest at night. OPP differences between animals are significant, which may put the NHPs with lower OPPs at risk for ischemic damage at lower IOP levels.

Oxygen modulation of flicker-induced blood flow changes in the human retina

Leopold Schmetterer^{1,2}, Stefan Palkovits¹, Gerhard Garhöfer¹,

¹*Department of Clinical Pharmacology, Medical University of Vienna*

²*Center of Medical Physics and Biomedical Engineering, Medical University of Vienna*

Purpose: There is conflicting evidence whether neurovascular coupling is altered by the level of oxygen in tissue. In the present study we investigated whether flicker-induced blood flow changes in the retina are influenced by breathing gas mixtures with different fractions of O₂ (FiO₂).

Methods: A total of 24 healthy subjects were included in this study. Retinal blood flow was studied by combining measurement of retinal vessel diameters using the Retinal Vessel Analyzer (RVA) with measurements of retinal blood velocity using laser Doppler velocimetry. Oxygen saturation was measured using reflectometry and oxygen extraction was calculated. Flicker-induced changes in retinal blood flow were studied during breathing room air and gas mixtures with 100%, 12% and 15% FiO₂.

Results: Flicker stimulation increased retinal blood flow retinal oxygen extraction ($p < 0.01$ each). During 100% oxygen breathing the response of retinal blood flow and retinal oxygen extraction was increased ($p < 0.01$ each). By contrast breathing gas mixtures with 12% and 15% FiO₂ did not alter flicker –induced retinal hemodynamic changes.

Conclusions: In humans 100% oxygen breathing increases the retinal blood flow response to flicker-stimulation and also increases retinal oxygen extraction. The mechanism underlying this effect is unknown.

Retinal oxygen metabolism in healthy subjects

Doreen Schmidl^{1,2}, Stefan Palkovits^{1,3}, Gerhard Garhofer¹, Leopold Schmetterer^{1,2}

¹Department of Clinical Pharmacology, Medical University of Vienna, Austria

²Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Austria

³Department of Ophthalmology, Medical University of Graz, Austria

Purpose: The aim of the present studies was to determine retinal oxygen extraction (ROE) by combining measurements of retinal blood flow with retinal oxygen saturation in healthy subjects during normoxia as well as during breathing of different gas mixtures to gain more insight in the physiology of ROE.

Methods: All studies were performed in healthy volunteers. Oxygen saturation in retinal vessels was measured using a commercially available dynamic vessel analyzer (DVA, Imedos, Germany) extended with a spectrophotometric module for measurement of oxygen saturation. In addition, retinal vessel diameters were determined using the same device and blood flow velocities were assessed with a laser Doppler velocimeter (LDV). Systemic blood oxygen tension was obtained from arterialized blood samples drawn from the earlobe. Measurements were performed during normoxia as well as during breathing 100% oxygen, a mixture of 15% oxygen and 85% nitrogen and a mixture of 12% oxygen and 88% nitrogen.

Results: During hyperoxia, a significant decrease in ROE by as much as $62.5\% \pm 9.5\%$ ($P < 0.001$) was observed. In contrast, during graded hypoxia, ROE did not change (12%O₂: $-2.8 \pm 18.9\%$, $p = 0.65$; 15%O₂: $2.4 \pm 15.8\%$, $p = 0.26$).

Conclusions: Our results indicate that during systemic hypoxia, ROE remains constant in healthy humans. In contrast, during hyperoxia, a large amount of oxygen consumed by the inner retina seems to derive from the choroid, which is supported by several animal studies. This should be taken into account when, interpreting oxygen saturation data in retinal arteries and veins without quantifying blood flow.

Specific topic: Ocular ischemia and blood flow

Corresponding author: leopold.schmetterer@meduniwien.ac.at

Role of Neuronal Nitric Oxide Synthase in Regulating Retinal Blood Flow In Response to Flicker-Induced Hyperemia in Cats

Taiji Nagaoka

Department of Ophthalmology, Asahikawa Medical University, Asahikawa, JAPAN.

Purpose: To investigate how neuronal nitric oxide synthase (nNOS) contributes to regulation of the retinal circulation during rest and flicker stimulation in cats.

Methods: Using laser Doppler velocimetry, we measured the vessel diameter and blood velocity simultaneously and calculated the retinal blood flow (RBF) in feline first-order retinal arterioles. Following intravitreal injections of N ω -Nitro-L-arginine methyl ester (L-NAME), non-selective NOS inhibitor, and N ω -propyl-L-Arginine (L-NPA), a selective nNOS inhibitor, we continuously monitored the retinal circulation without any perturbations for 2 hours. We then examined the changes in the RBF in response to 16-Hz flicker stimuli for 3 minutes 2 hours after intravitreal injection of phosphate buffer saline (PBS) as a control, L-NAME, L-NPA, and thromboxane A2 (TXA2) analogue U46619 as basal tone-adjusted control.

Results: After intravitreal injection of L-NAME and L-NPA, the RBF decreased gradually in a dose-dependent manner. In the PBS group, the RBF increased gradually and reached a maximal level after 2 to 3 minutes of flicker stimuli. After 3 minutes of 16-Hz flicker stimuli, the RBF increased by $53.5 \pm 3.4\%$ compared with baseline. In the L-NAME and L-NPA groups, the increases in RBF during flicker stimulation were attenuated significantly compared with the PBS group. In the TXA2 group, the reduction in the increase in RBF during flicker stimuli was comparable to that in the PBS group.

Conclusions: The current results suggested that increased RBF in response to flicker stimulation may be mediated by NO production via nNOS activation.

Effect of AR-13324 on episcleral venous pressure (EVP) in Dutch Belted rabbits.

Jeffrey W. Kiel¹ and Casey C. Kopczynski²

¹Department of Ophthalmology, University of Texas Health Science Center at San Antonio, San Antonio, TX

²Aerie Pharmaceuticals, Inc., Research Triangle Park, NC

Purpose: AR-13324 is a new potential glaucoma drug that has been shown to lower intraocular pressure (IOP) by increasing trabecular outflow facility and decreasing aqueous production. The present study tested the hypothesis that AR-13324 also lowers IOP by reducing episcleral venous pressure (EVP).

Methods: In Dutch Belted rabbits (n=11), arterial pressure (BP), IOP, carotid blood flow (BF_{car}), heart rate (HR) and EVP were measured invasively. Animals were dosed with AR-13324 (0.04%, topical) once daily for three days. On Day 3, the animals were anesthetized and then measurements obtained prior to dosing with AR-13324 (n=6) or vehicle (n=5) and for 3 hours after dosing. The data (mean $\pm$ SE) were analyzed by repeated measures ANOVA with post hoc testing. Retrospective baseline data from prior similar studies in New Zealand White rabbits were also compiled.

Results: Baseline values were: BP; 101 ± 3 mmHg, IOP; 33 ± 3 mmHg, EVP; 16 ± 1 mmHg, BF_{car}; 41 ± 4 ml/min, and HR; 330 ± 6 bpm. Three hours after AR-13324 dosing, IOP was reduced by $39 \pm 7\%$ ($p < 0.001$) and EVP by $35 \pm 4\%$ ($p < 0.05$); after vehicle dosing, IOP was reduced by $24 \pm 4\%$ ($p < 0.05$) and EVP increased by $25 \pm 5\%$ ($p < 0.05$). BP, BF_{car} and HR were unchanged.

Conclusions: AR-13324 lowers EVP significantly in Dutch Belted rabbits. Also noteworthy, the baseline values for BP, IOP, EVP, BF_{car} and HR in the Dutch Belted rabbit are higher than in the New Zealand rabbit.

Retinal Ganglion Cell Neuroprotection by JNK inhibition and Neuritin-1 Gene Therapy

Abbot F. Clark^{1,2}, Byung-Jin Kim^{1,2}, Tasneem P. Sharma^{1,2}, Yang Liu^{1,2}, Iok-Hou Pang^{2,3}

¹Cell Biology & Immunology, U. North Texas Health Science Center, Ft. Worth TX, USA

²North Texas Eye Research Institute, UNTHSC, Ft. Worth TX, USA

³Pharmaceutical Sciences, UNTHSC, Ft. Worth TX, USA

Purpose: A number of blinding ocular diseases, including glaucoma, involve retinal ganglion cell (RGC) death, leading to irreversible vision loss and blindness. Better understanding the molecular pathogenic pathways for RGC neurodegeneration will lead to improved neuroprotective therapeutic strategies.

Methods: We used 2 acute mouse models of RGC damage; optic nerve crush (ONC) and retinal ischemia/reperfusion I/R injury. The following was conducted at weekly intervals after injury: retinal gene expression profiling, immunohistochemistry, and western immunoblotting; spectral domain-OCT and scotopic ERG to assess retinal structure and function; and histological assessment of the retina and superior colliculus (SC). Varying doses of the JNK inhibitor SP600125 were administered daily in the retinal I/R model. AAV2.NRN1 gene therapy (ivt injection 14 days prior to injury) was administered in the ONC model.

Results: Retinal pJNK and pJUN expression was significantly increased within hours of I/R injury. Treatment with all three doses of the JNKi totally suppressed I/R induced thinning of the inner retinal, loss of cells in the RGC layer, decreased ERG b-wave amplitudes, and neuronal loss in the contralateral SC. Retinal expression of NRN1 was progressively and significantly decreased after ONC injury. Transduction of RGCs with AAV2.NRN1 prior to ONC significantly reduced RGC loss and provided total functional protection in the pSTR ERG response.

Conclusions: JNK inhibition morphologically and functionally protected the visual axis from I/R injury. NRN1 gene therapy partially protected RGCs from optic nerve crush injury. Both JNK and NRN1 should be further explored as new RGC neuroprotection therapeutic targets.

Predictable candidate genes for glaucoma gene therapy.

Terete Borrás, *University of North Carolina at Chapel Hill*

Purpose: To select functional candidate genes to develop as gene therapy drugs for glaucoma.

Methods: Cross-laboratories trabecular meshwork microarray analyses were performed for a first selection of relevant genes based on abundance, response to mechanical strain, glaucoma associated drugs and differential glaucoma expression. Genes involved in mechanisms known to affect IOP were assayed in sheep and rat elevated IOP models using scAAV2 viral vectors. Generation of a knock-in-Cre mouse and crosses with a floxed LacZ reporter line were conducted to determine the spatial and temporal distribution of a selected candidate gene.

Results: We have generated a list of 50 selected functional biomarkers and categorized them by the number of glaucomatous conditions that affected their expression. The three selected translational genes were MMP1, dominant-negative RhoA kinase (dnRhoA) and MGP. An intracameral single dose of scAAV2.MMP1 reduced the steroid-induced elevated in sheep for 3 weeks. An intracameral single dose of scAAV2.dnRhoA prevented the nocturnal elevation in rats for 4 weeks. One month old offspring from an Mgp.KI-Cre mouse crossed with an R26R.LacZ reporter line revealed intense Mgp-Cre mediated expression only in the trabecular meshwork and peripapillary sclera (ppSC).

Conclusions: Genes delivered to the eye's anterior segment represent a potential long-term and safe drug for the treatment of glaucoma. Because of his high specific expression in the trabecular meshwork and ppSC, the MGP's anti-calcification/anti-stiffness properties in the vascular tissue might translate into a potential dual targeting for glaucoma.

Disease Modifying/ Reversing Anti-Glaucoma Drugs: A High-Throughput Method for Lead Discovery

Jenny W. Wang¹, David F. Woodward¹ and W. Daniel. Stamer²

¹Allergan, Inc. Dept. of Biological Sciences, Irvine, CA, U.S.A.

²Duke University Medical Center, Department of Ophthalmology, Durham, N.C., U.S.A.

Purpose: Disease modification of the glaucoma disease requires a different approach to drug design. The end-point and cell based methodology should represent morphological events known to occur during disease progression. The preliminary stage of model development described herein involved a well-described biomechanical cell alteration.

Methods: The high-throughput cell impedance assay is a technique previously used to provide a cellular basis for the activity ocular hypotensive agents. By employing endothelial cells of Schlemm's-canal from normal and glaucomatous human eyes, disease induced biomechanical alteration was compared in response to drugs that would decrease or increase electrical impedance through a cell monolayer.

Results: By comparing ocular hypotensive drugs that would produce cytoskeletal relaxation or contraction, the clearest difference between normal and glaucomatous cell responses was achieved with the prostanoid FP receptor agonist, 17-phenyl PGF₂α.

Conclusions: These preliminary studies indicate the use of cell impedance for investigating and monitoring disease induced cellular alterations. This approach would be of particular use for drug discovery projects where high-throughput screening is essential, for example in epigenetic based research. Preliminary studies suggest 17-phenyl PGF₂α as a useful “tool” drug. However, transforming growth factor αα(TGFα) produced a profound response in these cells and may provide a more relevant implement, since it is actually implicated in the development of glaucoma.

Novel antiglaucoma therapies that target endothelial cell contraction

Ramaswamy Krishnan¹, Chan Young Park², Enhua H. Zhou², Dhananjay Tambe², Bohao Chen³, Tera Lavoie³, Maria Dowell³, Anton Simeonov⁴, David J. Maloney⁴, Aleksandar Marinkovic², Daniel J. Tschumperlin², Stephanie Burger², Matthew Frykenberg², James P. Butler², W. Daniel Stamer⁵, Mark Johnson⁶, Julian Solway³, Jeffrey J. Fredberg²

¹99 Brookline Avenue, Center for Vascular Biology Research, Beth Israel Deaconess Medical Center, Boston, MA 022152

²665 Huntington Avenue, Molecular and Integrative Physiological Sciences, Harvard School of Public Health, Boston, MA 02115

³5841 S. Maryland Avenue, Departments of Medicine, University of Chicago, Chicago, IL 60637

⁴6701 Democracy Boulevard, National Center for Advancing Translational Sciences, National Institutes of Health, Bethesda, MD 20892

⁵2351 Erwin Road, Duke Eye Center, Duke University, Durham, NC 27710

⁶2145 Sheridan Road, Department of Biomedical Engineering, Northwestern University, Evanston, IL 60208

Purpose: In glaucoma, current drug treatments and surgical treatments target reduction of intraocular pressure, but many patients remain refractory to those treatments. Because excessive contraction of the endothelial cells of the Schlemm's canal has been implicated recently in the etiology of glaucoma, we screened the Prestwick Chemical Library[®], comprised of 1,120 drugs already approved by the FDA or European Medicines Agency, to identify which among these might be an unanticipated candidate to relax SC cells in glaucoma.

Methods: We developed a novel high-throughput assay based upon Fourier-transform traction microscopy for direct quantitative measurements of SC contractile force, which serves as the targeted physiological endpoint.

Results: 17 individual drugs were identified as hits that blunted SC cell contraction. One was a toxin (Sanguinarine), 9 were β -adrenergic receptor agonists already well-known as SC relaxants, and one was a vasodilator (Alprostadiol). Alprostadiol was the most potent of these hits; further inspection revealed that alprostadiol reduced contractile force of SC cells in a dose-dependent manner and its efficacy was further validated in perfusion studies testing aqueous humor outflow function in enucleated mouse eyes.

Conclusions: Using cellular force as the target, here we screened the Prestwick Chemical Library[®] and identified novel drug candidates to blunt SC endothelial cell contraction in the context of glaucoma.

Steroid-Induced Glaucoma in Mice: A Model with Potential for Studying Disease Modification /Reversal

David F. Woodward^{1,2}, Darryl R. Overby², Jacques Bertrand², Elke Lütjen-Drecoll³, W. Daniel Stamer⁴

¹Allergan Inc., Department of Biological Sciences, Irvine, CA, U.S.A.

²Imperial College of London, Department of Bioengineering, London, England

³University of Erlangen-Nürnberg, Department of Anatomy II, Erlangen, Germany

⁴Duke University Medical Center, Department of Ophthalmology, Durham, NC U.S.A.

Purpose: Current glaucoma medications are designed to reduce intraocular pressure (IOP) and thereby slow or arrest neurodegenerative progression of the disease. Future anti-glaucoma therapeutics will likely focus on disease reversal rather than halting disease progression. To more closely examine pathology at the level of the conventional outflow tract, an animal model that replicates the etiology and morphological features of the glaucoma was investigated.

Methods: Steroid induced glaucoma in mice was used as a model. Subchronic dexamethasone was administered daily. IOP was measured pneumotonometrically and outflow facility was determined by stepwise perfusion of ex vivo eyes. Morphological studies were performed using light and transmission electron microscopy.

Results: Dexamethasone increased IOP by 2.6 ± 1.6 mmHg and decreased outflow facility by 52%. The length of the basement membrane underlying Schlemm's canal was significantly increased by subchronic dexamethasone treatments, which correlated with decreased outflow facility. Further morphological changes included fibrillar material in the trabecular meshwork, plaque-like material surrounding the elastic fibers, and scleral fibroblast to myofibroblast transformation.

Conclusions: Dexamethasone induced glaucoma in mice directly mimics morphological features of steroid induced glaucoma in man and partially mimics primary open angle glaucoma. The model appears well-suited for studying putative etiological factors, and gene, epigenetic, and disease modifying therapies.

Poster Sessions

Retinal Pharmacology

Aqueous Humor dynamics /Blood Flow

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Retinal Pharmacology

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Poster Abstracts

Selective multi-kinase inhibitor of ROCK/JAK/PDGFR- β reduces laser-induced CNV in rats

Cheng-Wen Lin, Jill M. Sturdivant, Mitchell A. deLong and Casey C. Kopczynski

Aerie Pharmaceuticals Inc., Research Triangle Park, NC, USA

Purpose: Rho-associated protein kinase (ROCK), Janus kinase (JAK), and platelet-derived growth factor receptor beta (PDGFR- β) have been implicated in the development of choroidal neovascularization (CNV). This study seeks to identify and evaluate selective multi-kinase inhibitors of ROCK/JAK/PDGFR- β for their ability to inhibit CNV. CNV is a hallmark of wet age-related macular degeneration (AMD).

Methods: 184 compounds from a library of ROCK inhibitors were screened at 500nM for activity against a panel of 456 human kinases. Subsequently, AR-13154 was selected for further investigation in a rat laser-induced CNV model. After retinal laser treatment on Day 0, AR-13154 (0.06, 0.6, or 6 μ g/mL) or vehicle was administered by intravitreal (IVT) injection on Days 1, 4, and 10 (n=5 rats/group). Aflibercept (800 μ g/mL) was administered by IVT injection on Day 1 as a positive control. The animals were sacrificed on Day 21, and the retinas were flat-mounted, stained with DAPI (nuclear) and Isolectin IB4-Alexa 594 (endothelial cells and microglia). CNV lesion sizes were measured using Image-Pro Plus.

Results: At 500nM, AR-13154 inhibited ROCK2, JAK2, JAK3, and PDGFR- β by >99%, 72%, 97%, and 89%, respectively. In flat-mount retinas, 6 μ g/mL of AR-13154 reduced mean CNV lesion size by 35% (P<0.001) while vehicle control and two lower doses of AR-13154 had no effect (p>0.05). Aflibercept (800 μ g/mL) reduced mean CNV lesion size by 23% (p<0.05).

Conclusions: AR-13154, a selective multi-kinase inhibitor of ROCK/JAK/PDGFR- β , significantly inhibited CNV in a rat model. AR-13154 merits further study as a potential treatment for wet AMD, either as monotherapy or in combination with anti-VEGF agents.

Molecular features of interaction between VEGF-A and anti-angiogenic molecules: a biophysical computational study.

Chiara Bianca Maria Platania¹, Luisa Di Paola², Salvatore Salomone¹, Gian Marco Leggio¹, Filippo Drago¹, Claudio Bucolo¹

¹*Department of Biomedical and Biotechnological Sciences. University of Catania, V.le Andrea Doria 6, 95125, Catania, Italy.*

²*School of Engineering, University CAMPUS BioMedico, Via A. del Portillo, 21, 00128 Roma, Italy*

Keywords: VEGFA, angiogenesis, molecular modeling.

Purpose: To analyze at atomistic level the energetic features of well characterized anti-angiogenic agents by means of computational approaches.

Methods: We carried out molecular modeling of aflibercept binding domain (VEGFR1d2_R2d3). We further modeled loops of ranibizumab and Fab-bevacizumab. Three replicas of all-atom molecular dynamics simulations of VEGFR1d2_R2d3, ranibizumab and Fab-bevacizumab and corresponding complexes with VEGFA were carried out. Binding energies of anti-angiogenic/VEGFA complexes have been predicted with MM-PBSA calculations.

Results: Protein-protein binding is strongly influenced by ionic strength, thus it is reported that greater electrostatic stabilization energy of a complex is associated to faster K_{on} . Papadopoulos et al. (2012) reported a higher K_{on} , upon binding with VEGFA, for aflibercept compared to ranibizumab and bevacizumab; we confirmed that VEGFR1d2_R2d3/VEGFA is stabilized by electrostatic energy. Furthermore, hydrophobic effect is one of the driving forces of protein-protein binding. Apolar desolvation energy accounts to hydrophobic effect, indeed we found that the apolar contribution to desolvation energy was correlated ($R^2 = 0.90$, $p = 0.001$) with experimental KD of analyzed complexes. The low experimental K_{off} of ranibizumab has been explained with higher number of stable contacts, H-bonds and lower conformational fluctuation compared to VEGFR1d2_R2d3 and Fab-bevacizumab bound to VEGFA. Furthermore we carried out molecular modeling of aflibercept binding domain bound to PlGF1 and VEGFB.

Conclusions: Molecular modeling approaches are feasible to evaluate binding features of VEGFA in complex with binding domains of anti-angiogenic drugs, indeed it is useful for characterization of protein-protein complexes. This approach may be useful to develop more effective anti-VEGF agents.

Anti-C5a therapy blocks systemic and local monocytes and protects RPE cells from damage in the *Cfh*^{+/~}HFC model of AMD

Christopher B. Toomey^{1,2}, Una Kelly¹, Dan Saban^{1,3} and Catherine Bowes Rickman¹

¹Department of Ophthalmology

²Department of Cell Biology

³Department of Immunology Duke University Medical Center, Durham, NC, USA

Purpose: Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly in developed countries and there are no therapies for the majority of patients. A major contributing mechanism to AMD development is complement dysregulation. Furthermore, monocytes have been shown to be involved in AMD; however, the role monocytes play in the pathogenesis of the disease and the pharmacologic potential of blocking monocyte recruitment is unclear. We hypothesize that chemoattractant, C5a, plays a major role in recruiting monocytes and AMD pathology.

Methods: In order to determine the role of C5a and monocyte recruitment in AMD-like pathology in old complement factor H heterozygous (*Cfh*^{+/~}) mice fed a high fat, cholesterol-enriched (HFC) diet (*Cfh*^{+/~}~HFC), *Cfh*^{+/~}~HFC mice were given weekly intraperitoneal injections of 30mg/kg anti-C5a (4C9, Pfizer). Extravascular flow cytometry, RPE flat mounts and electroretinograms were used to determine the role of anti-C5a treatment on the immune cell populations and AMD-like pathology.

Results: Anti-C5a treatment blocks classical and non-classical monocytoysis and the increase in a CD64⁺ population in the extravascular RPE/Choroid. Furthermore, anti-C5a treatment ameliorates the RPE dysmorphogenesis and visual function decline seen in the *Cfh*^{+/~}~HFC model.

Conclusions: Our results show local CD64⁺ monocyte recruitment occurs in a C5a dependent manner in the *Cfh*^{+/~}~HFC model of AMD and that these monocytes play a role in the pathophysiology of the disease. These results support the notion that protecting RPE cells from the pathological effects of complement activation is a fruitful therapeutic avenue for AMD.

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Trichostatin A Rescues RPE Function in Diabetic Rats

Danielle Desjardins¹, Yueying Liu¹, Philip Yates¹, Craig Crosson¹, Zsolt Ablonczy¹

¹Ophthalmology, Medical University of South Carolina, Charleston, SC, United States.

Purpose: The etiology of diabetic macular edema has been tied to endothelial dysfunction of the inner blood-retina barrier. However, recent studies have shown that dysfunction of the retinal pigment epithelium (RPE) also plays a role in this disease. Changes in protein acetylation have been shown to play a central role in pathophysiological responses to hyperglycemia. In this study, we investigate the effects of the HDAC inhibitor trichostatin A (TSA) on hyperglycemia-induced RPE dysfunction.

Methods: Brown Norway rats (130-150g) were injected IP with 60 mg/kg of streptozotocin (STZ, citrate buffer). Animals were considered diabetic with plasma glucose of 250 mg/dL or greater. Retinal thickness, plasma glucose, and weight were measured. Animals were treated with TSA (2.5 mg/kg BID IP, 10% DMSO) or vehicle for 4 days before bleb injection. Subretinal blebs (1 μ L PBS) were created and resorption observed and measured via optical coherence tomography and rates of RPE fluid resorption calculated.

Results: In normoglycemic rats, resorption rate was 8.92 ± 1.19 $\mu\text{L} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ (n=4). Nine weeks after diabetes induction, resorption reduced to 2.43 ± 0.55 $\mu\text{L} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ ($p < 0.05$, n=6). In diabetic rats, TSA treatment increased fluid resorption to 8.11 ± 1.54 $\mu\text{L} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$ (n=7). In control rats TSA had no significant effect on resorption (8.068 ± 1.81 $\mu\text{L} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$; n=4).

Conclusions: Acute TSA treatment normalized RPE fluid resorption in diabetic animals. These data provide evidence that decreases in protein acetylation plays a role in hyperglycemia-induced RPE dysfunction. However, it is not clear if the use of HDAC inhibitor alone will be efficacious in treating diabetic macular edema.

Iron chelation by transferrin preserves vision in retinal degeneration models

Emilie Picard¹, Marianne Berdugo¹, Mohammed El Sanharawi¹, Quentin Le Rouzic¹, Laurent Jonet¹, Marie-Christine Naud¹, Jean-Claude Jeanny¹, Yves Courtois¹, Francine Behar-Cohen¹

¹*Team 17, UMRS 872, Centre de Recherche des Cordeliers, Paris, France.*

Purpose: Iron has a dual role. Essential for cellular functions, it enhances oxidative stress when it is in excess and under a labile form. Iron accumulation is observed in outer retina of patients with Age-Related Macular Degeneration and in animal models of retinal degeneration like light-damage or inherited genetic rodents. Here, we demonstrate the protective role of local delivery of Transferrin (Tf), an endogenous iron chelator, on rat model light-induced photoreceptors degeneration.

Methods: Retinal degeneration was light-induced with 24 hours of intense white light-emitting diodes exposure. Tf was delivered by intravitreal (IVT) injection before or after light exposure. Twelve days later, in vivo exploration were done by Optical coherence tomography and retinal electrophysiological responses by electroretinography (ERG; scotopic and photopic recordings). Then, retinal sections were realized to evaluate outer nuclear layer thickness, iron-related proteins expression and inflammation responses.

Results: IVT injection displays Tf presence overall the retina for several hours. Light exposure induces a destruction of retina at the superior pole and at a lesser manner at the inferior pole. IVT of Tf just before light exposure preserved cones and rods histology, and more than 50% of photoreceptors layers, and electrophysiological retinal functions. IVT injection just after light exposure protects partially photoreceptors layer on inferior pole. Tf also preserves iron homeostasis and decreases inflammatory cells responses.

Conclusion: Iron chelation strategy demonstrates a powerful protective effect playing on cells death and retinal homeostasis. Transferrin has high therapeutic potential on neurodegenerative processes.

Inflammatory Markers and Angiogenic Mediators in Patients with Age Related Macular Degeneration from Pakistan

Fareeha Ambreen, Irfan Z. Qureshi

Purpose: To determine association of serum angiogenic mediators and inflammatory markers with age related macular degeneration (AMD) in Pakistani patients.

Methods: The study was case-control, cross-sectional. Out of total 3911 patients screened for ophthalmic complications, ninety were diagnosed with AMD. One hundred healthy age-matched control subjects were recruited. The confirmation of AMD was carried out through slit lamp examination, fundoscopy and ocular coherence tomography.

Serum apolipoprotein E (apoE), leptin, HTRA1, interleukin 6 (IL-6), IL-8, vascular endothelial growth factor (VEGF), C-reactive protein (CRP) and complimentary factor H (CFH) were estimated serum samples of all subjects. Data were compared with Mann-Whitney U Test. Pearson's correlation demonstrated correlation among above factors, while logistic regression predicted the risk factors involved in AMD. The significance level was $p < 0.05$.

Results: Significantly elevated serum apoE ($p < 0.008$), HTRA1 ($p < 0.0001$), IL-6 ($p < 0.0193$), IL-8 ($p < 0.0001$), VEGF ($p < 0.0001$) and CRP ($p < 0.0001$) concentrations were observed in AMD patients, while serum leptin ($p < 0.001$) and CFH ($P < 0.0001$) concentrations showed significant decrease. Logistic regression demonstrated elevated HTRA1 ($p < 0.0001$), IL-6 ($p < 0.006$), IL-8 ($p < 0.002$), VEGF ($p < 0.037$) and CRP ($p < 0.005$) with lowered CFH ($p < 0.0001$) appearing to be the risk factors for AMD.

Conclusion: The present study demonstrates that inflammatory markers and angiogenic growth factors are significantly altered in AMD patients irrespective of the fact that the patients belonged to a geographically and racially distinct Pakistani population.

Platelet factor-4 variant (CXCL4L1/PF-4var) antagonizes VEGF functions and inhibits diabetes-induced blood-retinal barrier breakdown

Ghulam Mohammad, *Department of Ophthalmology, College of Medicine, King Saud University, Riyadh, Saudi Arabia.*

Purpose: To investigate the expression of platelet factor-4 variant (CXCL4L1/PF-4var) in epiretinal membranes from patients with proliferative diabetic retinopathy (PDR) and to evaluate the role of PF-4var in the regulation of blood-retinal barrier (BRB) breakdown in diabetes with the use of a diabetic rat model and cultures of human retinal microvascular endothelial cells (HRMEC).

Methods: Epiretinal membranes from 14 patients with PDR were studied by immunohistochemistry. Diabetic rats were treated intravitreally with PF-4var or the anti-vascular endothelial growth factor (VEGF) agent bevacizumab on the first day after diabetes induction. The contralateral eye received intravitreal injection of phosphate buffer saline as the paired control. BRB breakdown was assessed *in vivo* with fluorescein isothiocyanate (FITC)-conjugated dextran and *in vitro* in HRMEC by transendothelial electrical resistance and FITC-conjugated dextran cell permeability assay. Occludin, VE-cadherin, HIF-1 α , VEGF, TNF- α , RAGE, caspase-3 levels and generation of reactive oxygen species (ROS) were assessed by Western blot, enzyme-linked immunosorbent assays or spectrophotometry.

Results: In epiretinal membranes, vascular endothelial cells and stromal cells expressed PF-4var. *In vitro*, HRMEC produced PF-4var after stimulation with a combination of IL-1 β and TNF- α and PF-4var inhibited VEGF-mediated hyperpermeability in HRMEC. In rats PF-4var was as potent as bevacizumab in attenuating diabetes-induced BRB breakdown. This effect was associated with upregulation of occludin and VE-cadherin and downregulation of HIF-1 α , VEGF, TNF- α , RAGE and caspase-3, whereas ROS generation was not altered.

C

onclusions: Our findings suggest that increasing intraocular PF-4var levels early after the onset of diabetes protects against diabetes-induced BRB breakdown.

Inhibition of Thyroid Hormone Receptor Protects Cone Photoreceptors in Retinal Degeneration

Hongwei Ma¹, Michael Butler¹, Joshua Belcher¹, T. Michael Redmond², Thomas S. Scanlan³ and Xi-Qin Ding¹

¹*Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK*

²*Laboratory of Retinal Cell and Molecular Biology, National Eye Institute, Bethesda, MD*

³*Department of Physiology and Pharmacology, Oregon Health & Science University, Portland, OR*

Purpose: Thyroid hormone (TH) signaling regulates cell proliferation, differentiation, and apoptosis. In the retina, TH signaling plays a central role in cone opsin expression and patterning. We previously showed that suppressing TH signaling by anti-thyroid treatment preserves cones in mouse models of retinal degeneration. This work investigates whether inhibition of TH receptors (TRs) affects cone viability in retinal degeneration.

Methods: The cone precursor Weri RB-1 cell line was used to determine the antagonistic activity of the TR antagonists, NH3 and 1-850. M-opsin, S-opsin, and TR β 2 expression levels were analyzed by qPCR following T3 treatment in the presence and absence of TR antagonists. The severe cone degeneration mouse model, Rpe65^{-/-}, was used to determine whether treatment with TR antagonists protects cones. NH3 or vehicle was delivered by intravitreal injection at postnatal day 5 (P5), and retinas and eyes were collected on P24. 1-850 or vehicle was injected intraperitoneally daily from P5 to P29, and retinas and eyes were collected on P30. Cone survival was evaluated by examining cone density by immunohistochemical approaches.

Results: Treatment with T3 increased M-opsin and decreased S-opsin expression in Weri cells. The effects of T3 treatment were inhibited by NH3 and 1-850 in a dose-dependent pattern. Cone density in Rpe65^{-/-} mice increased about 35% following NH3 and 1-850 treatment.

Conclusion: We show that treatment with TR antagonists systemically or ocularly improved cone survival in mouse model. Our findings suggest that suppression of TR signaling locally in the retina may represent a novel strategy for retinal degeneration management.

Support: This work was supported by grants from the National Eye Institute (P30EY12190, R01EY019490, R21EY024583, and T32EY023202) and the Foundation Fighting Blindness.

Gossypol Acetic Acid protects RPE cells from oxidative stress-induced necrosis

Jakub Hanus¹, Shusheng Wang^{1,2}

¹ Department of Cell and Molecular Biology, Tulane University, New Orleans, LA, USA

² Department of Ophthalmology, Tulane University, New Orleans, LA, 70118, USA

Purpose: Age-related macular degeneration (AMD) is the leading cause of severe vision loss in people aged over 50. It is multi-factorial disease with unclear etiology. Age is the most consistent risk factor associated with AMD, genetic factors, oxidative stress and inflammation. We recently conducted systematic analysis of RPE cell death in response to oxidative stress, and found cardinal features of necrosis. Since dry AMD remains without any treatment available we screened for FDA-approved natural compounds that prevent oxidative stress-induced RPE necrosis.

Methods: ARPE-19 cells were treated with Gossypol Acetic Acid (GAA) followed by exposure to hydrogen peroxide. Cell survival was measured with MTT, necrosis markers were analyzed by RIPK3 activation, HMGB1 release, and ATP level. Gene expression was analyzed by qPCR and functional studies were performed using siRNA technology.

Results: We identified GAA as an effective inhibitor of ARPE-19 oxidative stress-induced necrosis. It exclusively inhibits necrotic intrinsic pathway and prevents RIPK3 activation. It is a potent antioxidant that inhibits ROS accumulation, presumably through regeneration of overoxidized peroxiredoxins. Its antioxidant properties are mediated through upregulation of Sestrin2. We further confirm role of Sestrin2 in response to oxidative stress and show that upregulation of Sestrin2 effectively prevents accumulation of ROS in cells exposed to oxidative stress. We found that GAA regulates Sestrin2 expression through FoxO3 transcription factor.

Conclusions: GAA is a potent inhibitor of oxidative stress-induced RPE necrosis by regulating FoxO3/SESN2 pathway. This study may have significant therapeutic implications in treating age-related diseases.

In Vivo Imaging of Retinal Caspase Activity with the Novel Near Infrared Dye: 780-VAD-FMK

Jeffrey A Jamison¹, Gary L Johnson²

¹*Ophthy-DS, Inc. Kalamazoo, MI,*

²*Intracellular Technologies, Minneapolis, MN*

Purpose:

Apoptosis is the primary death pathway in disease such as AMD, Diabetic Retinopathy, Retinal Detachment, and Glaucoma. Models of these conditions exhibit complications such as extended development of pathology, variability of induced disease, or lack of sensitive *in-vivo* endpoints. This increases drug development costs and limits productivity. A method to evaluate apoptosis *in-vivo* would enhance disease monitoring, provide a quick assay of efficacy, and allow pre-screening of subjects for study inclusion.

780-VAD-FMK is a low molecular weight infrared probe designed for use in oncology which binds to active caspases and accumulates transiently within the cells. Unbound material is quickly washed out and eliminated by normal physiological processes. The purpose of this study is to evaluate 780-VAD-FMK for use in ocular disease.

Methods:

Blue Light Damage is used to screen molecules for treatment of geographic atrophy. Caspase 3 is expressed in retina 1-3 days post light exposure. Rats were exposed to intense blue light on Day 0. On Day 2 animals received 100 nM/kg of 780-VAD-FMK or vehicle intravenously and were imaged using the Heidelberg Spectralis.

Results:

Animals receiving vehicle had no detectable activity. 780-VAD-FMK animals without light exposure had faint diffuse activity. Animals receiving 780-VAD-FMK with light exposure had an intense pattern of staining consistent with known retinal atrophy.

Conclusions:

It is possible to image caspase activity in live animals using the 780-VAD-FMK probe and a commercially available retinal camera. Future work will include optimization of imaging time and dose as well as application to additional disease models.

The sigma 1 receptor ligand (+)-pentazocine ((+)-PTZ) preserves cone function in the Pde6rd10 (Rd10) mouse model of retinitis pigmentosa

Jing Wang^{1,2}, Alan Saul^{2,3}, Sylvia B. Smith^{1,2,3}

¹Department of Cellular Biology and Anatomy, Medical College of Georgia (MCG), Georgia Regents University (GRU), Augusta, GA

²James and Jean Culver Vision Discovery Institute, GRU, Augusta, GA

³Department of Ophthalmology, MCG, GRU, Augusta, GA

Purpose: $\sigma 1R$, a unique transmembrane protein localized to ER, mitochondria, nuclear and plasma membranes, has substantial neuroprotective properties. We asked whether (+)-PTZ, a high-affinity $\sigma 1R$ ligand, could attenuate rod-cone degeneration in Pde6rd10 mice, which demonstrate rapid, profound photoreceptor (PRC) loss.

Methods: Pde6rd10 mice were injected i.p. with (+)-PTZ (0.5 mg • kg⁻¹) every other day beginning at P14. Mice were subjected to ERG and OCT at P35. At P42, eyes were enucleated, cryosectioned and subjected to morphometric analysis and immunohistochemistry (IMH) to evaluate rods (anti-rhodopsin), cones (anti-cone-arrestin), rod bipolar cells (anti-PKC $\sigma 1R$) and Müller cell activation (anti-GFAP). Data were compared to age-matched wildtype (WT) and non-injected-Pde6rd10 mice.

Results: ERG showed partial rescue of scotopic (rod) responses in (+)-PTZ-injected versus non-injected-Pde6rd10 mice and significant rescue of photopic (cone) responses, especially b-wave amplitude (~50% of WT mice - compared to virtually no response in non-injected-Pde6rd10 mice). Remarkably, tests using “natural” noise photopic stimuli showed similar strength responses in (+)-PTZ-injected mice versus WT. OCT revealed increased retinal thickness in (+)-PTZ-injected versus non-injected Pde6rd10 mice, which was similar to the morphometric data: WT: 203.03±36.22 μ m; Pde6rd10: 84.02±35.65 μ m; (+)-PTZ-injected-Pde6rd10: 115.99±28.59 μ m. The number of PRC rows was significantly greater in (+)-PTZ-injected (2.3±1.1) versus non-injected Pde6rd10 mice (0.96±0.6). IMH showed preservation of cones and bipolar cells and decreased Müller cell GFAP levels in (+)-PTZ-injected versus non-injected-Pde6rd10 mice.

Conclusions: Early (+)-PTZ administration preserved cone function in Pde6rd10 mice suggesting that activation of $\sigma 1R$ may be a novel and promising protective strategy in severe retinal degenerations.

Use of machine learning-based high content methodologies to identify novel retinal cell phenotypes.

John A. Fuller¹, Cynthia A. Berlinicke¹, James Inglese^{2,3}, and Donald J. Zack^{1, 4, 5}

¹*Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD*

²*National Center for Advancing Translational Sciences, NIH, Rockville, MD*

³*National Human Genome Institute, NIH, Bethesda, MD*

⁴*Departments of Molecular Biology and Genetics, Neuroscience, and Institute of Genetic Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD*

⁵*Institut de la Vision, Paris 75012, France*

Purpose: High content analysis (HCA) has become a leading methodology in phenotypic drug discovery efforts. Typical HCA workflows include imaging cells using an automated microscope and analyzing the data using algorithms designed to quantify a specific phenotype of interest. Due to the richness of high content data, unappreciated phenotypic changes may be discovered in existing image sets using interactive machine-learning based software systems.

Methods: Primary postnatal day four murine retinal cells from the photoreceptor (PR) labeling QRX-EGFP reporter mouse were isolated, seeded, treated with a set of 234 profiled kinase inhibitors and then cultured for one week, and then images were acquired using high content imaging instruments (Acumen Explorer, InCell2000, Cellomics VTI). Images were analyzed with an open source HCA analysis tool, Phenoripper to identify the high GFP-inducing treatments that additionally resulted in the most diverse phenotype compared to the vehicle control samples .

Results: The pyrimidinopyrimidone kinase inhibitor, CHEMBL-1766490, a pan kinase inhibitor, whose major known targets are p38 α , and Src family member lck was identified as an inducer of photoreceptor neuritogenesis . Classification of this compound as neurite –promoting using a cell-independent image analysis tool, was corroborated using an alternate cell-based method of image analysis, (ThermoFisher Cellomics Neuronal Profiling), which measured quantitative differences in the mean neurite length in GFP cells treated with CHEMBL-1766490.

Conclusions: Interacting with data using machine learning algorithms may lead to the discovery of unexpected phenotypes following small molecule treatment.

Imprinting and epigenetic modulation of retinal disease in mice

Kacie J. Meyer^{1,2}, Michael G. Anderson^{1,2,3}

¹*Department of Molecular Physiology and Biophysics, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA*

²*Department of Ophthalmology and Visual Sciences, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, IA*

³*Center for the Prevention and Treatment of Vision Loss, VA Medical Center, Iowa City, IA*

Purpose: Epigenetics likely contributes to several genetically complex ocular diseases, including glaucoma and age-related macular degeneration. Genomic imprinting is an epigenetic phenomenon resulting in mono-allelic gene expression dependent on the parent-of-origin. Expression from imprinted genes is typically regulated through differentially methylated regions. The goal of this study is to identify imprinted genes in the mouse retina and determine the phenotypic effect of pharmacological manipulation of DNA methylation in a mouse model of retinal degeneration.

Methods: Using RNA-Seq to determine parent-of-origin expression, we analyzed the retinal transcriptome of two genetically diverse inbred mouse strains and their resulting F1 progeny from reciprocal crosses. To test the influence of DNA methylation on retinal disease, we fed a diet supplemented with Bisphenol-A (BPA), a compound known to result in DNA hypomethylation, to rd16 mutant mice and measured retinal thickness using OCT.

Results: To date, the data identify 25 known imprinted genes that are expressed and imprinted in the retina of mice. An analysis to identify novel imprinted retinal genes is ongoing. Progeny resulting from parents fed a diet supplemented with BPA have a significantly decreased retinal thickness compared with progeny on a control diet (123 μm vs. 128 μm , $p < 0.02$).

Conclusions: This work provides proof-of-principle evidence of a role for epigenetic mechanisms in retinal disease. Interestingly, several imprinted genes map to known glaucoma loci. Future studies will identify targets of BPA that contribute to its effect and testing how imprinted genes may contribute to causes and treatments for common eye diseases.

Carnosic acid slows photoreceptor degeneration in *Pde6rd10* mice by controlling oxidative stress and endoplasmic reticulum stress

Kai Kang, Matthew J. Tarchick, Xiaoshan Yu, Minzhong Yu

Department of Ophthalmic Research, Cole Eye Institute, Cleveland Clinic Foundation, Cleveland, OH

Purpose: Oxidative stress (OS) is the main factor underlying photoreceptor degeneration in animal models of retinitis pigmentosa (RP). Carnosic acid (CA) is an effective polyphenolic antioxidant, with the ability to prevent lipid peroxidation and biological membrane disruption by scavenging oxygen hydroxyl radicals and lipid peroxy radicals. Our aim was to evaluate the efficacy of CA in ameliorating retinal degeneration in the *Pde6rd10* mouse and to determine the underlying mechanism.

Methods: CA (15 mg/kg) or vehicle (canola oil) was administered by intraperitoneal injection to *Pde6rd10* mice daily from postnatal day (P) 6 to P20. At P21, animals were examined by electroretinography (ERG), TUNEL staining and immunohistochemical techniques.

Results: Comparing to vehicle-treated animals, mice treated with CA had larger amplitude ERGs under both dark- and light-adapted conditions ($p < 0.01$), fewer TUNEL-positive cells in the outer nuclear layer (ONL) and a thicker ONL (both $p < 0.01$). None of these measures, however, matched those of wild-type mice. Immunohistochemical staining showed that retinas of mice treated with CA had higher levels of activity in the Nrf2-ARE pathway, increased Nrf2 accumulation in cell nuclei within the ONL, attenuated markers of endoplasmic reticulum stress, and decreased expression of ER chaperones including phospho-PERK, CREB, and ATF6 (all $p < 0.01$).

Conclusions: CA treatment reduces OS and ER stress in the ONL which is associated with greater survival of *Pde6rd10* photoreceptors. CA is a potentially effective antioxidant for slowing the photoreceptor degeneration associated with RP.

Key words: Carnosic acid, Oxidative stress, Endoplasmic reticulum stress, Retinal degeneration, Retinitis pigmentosa

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Mechanism protection of dietary supplement enriched in antioxidants and omega-3 in progressive light-induced retinal degeneration model

Khaoula Ramchani-Ben Othman^{1,2}, David Cia¹, Christine Cercy¹, Nathalie Jacquemot¹, Mohamed Amri², Michel Doly¹, Isabelle Ranchon-Cole¹

¹University Clermont 1, UFR Pharmacie Laboratoire de Biophysique Neurosensorielle, UMR Inserm 1107, Clermont-Ferrand, F-63001 France

²University Tunis El Manar, Laboratory of Functional Neurophysiology and Pathology, Research Unit, UR/11ES09, Department of Biological Sciences, Faculty of Science of Tunis, Tunisia.

Purpose: we have shown that dietary supplement (DS-T1672) protected from progressive light-induced retinal degeneration (PLIRD) in vivo. In the present study, we investigated the involved mechanism.

Methods: Animals treated with water or DS were sacrificed in the dark (D0) or at different time during (Day (D) 1, D3 and D7) or after (D+1, D+3, D+7, D+14) PLIRD to evaluate twelve cytokines by milliplex. In vitro, ARPE-19 cells were treated with different concentration of DS (noted as DHA concentration) for one week before being stressed with H₂O₂ at 600 μ M for 2 hours. Cells viability was determined by MTT.

Results: During PLIRD, IL-12, IL-2 and IL-1 α significantly ($p < 0.03$) increased. Then, at D+1 all cytokines decreased excepted for IL-18 which is still increased by 200%. At D+14, IL-12 was significantly higher than at D0. In vitro, DS has no significant effect on ARPE-19 cells growth from 0.35 μ M DHA to 88 μ M DHA, but at 118 μ M DHA it induced a 36 % cells death. H₂O₂ at 600 μ M induced 40 % of ARPE-19 cells death in untreated cells but cells death is reduced when cells are pretreated with DS, by 20 % at the lowest concentration (0.35 μ M DHA) up to 31 % at 88 μ M DHA.

Conclusions: DS protects retinal pigment epithelium cells from oxidative-induced cell death. Pro-inflammatory cytokines are induced by light. IL-12's increase two weeks after PLIRD suggests that light initiates a chronic inflammation mechanism. Further experiments are on course to evaluate the effect of dietary supplement on these cytokines.

Keys words: progressive light-induced retinal degeneration; dietary supplement, cytokines, ARPE-19.

Gene based antioxidant therapy delays retinal degeneration in a mouse model of progressive retinal degeneration

Manas R Biswal¹, Christian Ildefonso¹, Haoyu Mao¹, Hong Li¹, and Alfred S Lewin¹.

¹*Molecular Genetics & Microbiology, University of Florida, Gainesville, FL*

Purpose: We established a model of RPE (retinal pigment epithelium) oxidative stress by Cre-lox mediated deletion of the Sod2 gene, that codes for the protective enzyme manganese superoxide dismutase (MnSOD), leading to features of geographic atrophy. We asked whether delivery of Sod2 using adeno-associated virus (AAV) could prevent retinal degeneration once it has begun and whether there is an optimal window of opportunity for such intervention.

Methods: Deletion of Sod2 was induced in mice with a “floxed” allele of Sod2 and an RPE-specific tet-transactivator controlling expression of cre. Retinal degeneration was monitored by electroretinography (ERG) and spectral domain optical coherence tomography (OCT) over a period of 9 months. Experimental mice at different ages received subretinal injection of a self-complementary AAV1 vector expressing mouse Sod2 cDNA in their right eyes and ScAAV1-GFP in left eyes.

Results: Following doxycycline induction of Cre, mice demonstrated signs of oxidative stress in RPE, a gradual decline in the ERG response and thinning of the outer nuclear layer which were statistically significant by 6 months. When injected early, ScAAV1-Sod2 delayed the progressive retinal degeneration in RPE specific Sod2 knockout mice.

Conclusions: Deletion of Sod2 in the RPE leads to some of the salient features of dry AMD. ScAAV1 delivery of Sod2 led to expression in RPE. Delivery of Sod2 vector can be used as a tool to reverse oxidative stress in this mouse model of dry AMD.

Long-term low-dose aspirin use promotes angiogenesis in laser induced choroidal neovascularisation

Mei Chen, Jiawu Zhao, Rosana Penalva, Heping Xu

Centre for Experimental Medicine, School of Medicine, Dentistry & Biomedical Science, Queen's University Belfast, Northern Ireland, UK

Purpose: Aspirin is widely used by the elderly to reduce the risk of heart attack and stroke. Clinical investigations have found that long-term use of low-dose aspirin is associated with the increased risk of neovascular age-related macular degeneration (nvAMD). The aim of this study was to establish an animal model to investigate the link between long-term aspirin use and nvAMD..

Methods: Two months old (n = 10) and 18 months old (n =10) C57BL/6J mice were gavage-fed daily with 1.5 mg /kg aspirin for 8 weeks. Control animals were fed with vehicle for 8 weeks. Laser-induced choroidal neovascularisation (CNV) was conducted and eyes were collected 7 days post-CNV induction. RPE/choroid flatmounts were stained with Isolectin B4 and examined by confocal microscopy. Immune cell activation in the blood was evaluated prior and after CNV induction by flow cytometry.

Results: The aged mice developed larger CNV compared to young mice (2.4-fold increment). Eight-week low-dose aspirin treatment significantly increased CNV in young mice but not aged mice. The percentage of CD3 T cell is decreased and CD11b monocyte is increased in aged mice compared to young mice. Aspirin treatment did not significantly affect the constitution of circulating immune cell.

Conclusions: Long-time low-dose aspirin increases CNV in young, but not old mice. The underlying mechanism remains to be elucidated.

Title: Ceramide Pathway, Novel Target for Retinal Degenerative Diseases

Nawajes Mandal^{1,2,3,6}, Megan Stiles^{1,6}, Eleanor Sun^{1,6}, Hui Qi^{1,6}, Jeremy Tan^{1,6}

¹Departments of Ophthalmology, ²Physiology, ³Oklahoma Center for Neuroscience, ⁴Medicine, ⁵Cell Biology, OUHSC, Oklahoma City, OK 73104, USA; ⁶Dean McGee Eye Institute, Oklahoma City, OK 73104, USA

Purpose: Major retinal degenerative diseases involve apoptotic photoreceptor cell death. Ceramide (Cer) is a known sphingolipid metabolite which works as a second messenger in the cell and can induce apoptosis through various mechanisms. We hypothesized that inhibiting ceramide synthesis in the cells can protect the retina from photoreceptor degeneration. FTY720 is a known low-toxicity immunomodulator that is the structural analog of sphingosine and can block de novo Cer production. In this study we tested the effects of systemic FTY720 in rat models of retinal degeneration.

Methods: We used two models, light induced retinal degeneration in Sprague Dawley (SD) rats and genetic mutant P23H rats. FTY720 was administered intraperitoneally before light damage, the dosage and temporal effect of FTY720 on the retina in vivo was measured by histological and functional analyses in light damaged model. P23H rats were dosed systemically with FTY720 from P15 to P35 days of age. Photoreceptor function by electroretinography (ERG), structural analysis by histology, Cer levels and molecular analyses of the retina were done at P21, P35 and P45.

Results: Retinal ceramide levels increased at various time points after light stress, well before active apoptosis. Systemic FTY720 prevented ceramide generation by the de novo pathway and protected retinal structure and function. Both retinal structure and function were significantly preserved in FTY720 treated P23H rats.

Conclusions: We conclude that ceramide level increases during photoreceptor degeneration in light induced and genetic models and inhibition of ceramide production prevents retinal degeneration in these models.

Conflict of Interest: Disclosed to OUHSC Office of Technology Transfer

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Chloroquine retinopathy as a modern model of macular degeneration

Néstor Más Gómez¹, Wennan Lu¹, Jason C. Lim¹, Alan M. Laties², Claire H. Mitchell^{1,3}

¹Anatomy and Cell Biology, ²Ophthalmology, and ³Physiology, University of Pennsylvania, Philadelphia, PA 19104.

Purpose: The lack of appropriate models for macular degeneration limits our ability to develop new treatments. The drug chloroquine can induce a bull's eye maculopathy involving strain to RPE cells and loss of photoreceptors. In animal models, chloroquine leads to an accumulation of lipofuscin-like material in the RPE and photoreceptor death. While these suggest parallels to more general forms of macular degeneration, modern markers of the disease, like lipid oxidation changes, have been examined. We have developed in vivo and in vitro models of chloroquine retinopathy to investigate parallels with macular degeneration.

Methods: For In vitro modeling, 10 μ M chloroquine was added to ARPE cells. Chloroquine retinopathy was induced in vivo by injecting 50 mg/kg IP 2x /week for 6 weeks.

Results: In vitro chloroquine increased particulate staining and autofluorescence. Autofluorescence was doubled by addition of photoreceptor outer segments, while retained opsin was greater with chloroquine. Levels of oxidized lipids and of HNE-adducted proteins were also increased in cells. Mice treated with chloroquine had increased TUNEL staining in photoreceptor nuclei and in lipids surrounding Bruch's membrane. qPCR indicated an increase in SOD2, while Elisa detected an increase in HNE-adducted proteins in the RPE/choroid, consistent with the increase found in vitro.

Conclusion: Together, these observations suggest that sustained delivery of low levels of chloroquine leads to a moderate chloroquine toxicity with markers of lipid oxidation associated with more general forms of macular degenerations to evaluate effectiveness of treatments.

sHSP Peptides Inhibit Protein Aggregation and Apoptosis in the Eye

Ram H. Nagaraj^{1,2}, Sruthi Sampathkumar¹ and Rooban B. Nahomi^{1,2}

¹Department of Ophthalmology & Visual Sciences, Case Western Reserve University, Cleveland, OH and ²Department of Ophthalmology, University of Colorado, Aurora, CO

Purpose: Small heat shock proteins (sHSP) are molecular chaperones and anti-apoptotic. Four peptides, one each in α A-, α B-crystallin, Hsp20 and Hsp27 have been identified to function similarly to the proteins they are derived from in their chaperone and anti-apoptotic activities. We have investigated whether these peptides could be used to inhibit cataract formation and ischemia/reperfusion (I/R)-induced retinal ganglion cell (RGC) death in mice.

Methods: Cataract was induced in 10-day-old rat pups by a single s.c. injection of sodium selenite. The peptides or their scrambled counterparts were injected at 10-50 μ g i.p. 6 hrs pre and 1, 2 and 4 days post sodium selenite injection. Cataract was assessed by slit lamp examination. Lens epithelial cell apoptosis in situ was assessed by TUNEL staining. Protein aggregation was assessed by extractability in buffer. I/R injury to retina in mice was induced by elevating intraocular pressure to 120-130 mm Hg for 30 min by cannulating with a 30-gauge needle attached to a line infusing normal saline, and reperfusion was established immediately. The acetyl derivatives of α A and α B-crystallin peptides or their scrambled counterparts were injected at 10 μ g i.p. twice daily for 4 days post I/R injury. After 10 days of I/R injury, NeuN immunostaining and TUNEL staining were used to determine apoptosis in the retinal ganglion cell layer.

Results: Intraperitoneal injection of the peptides or their lysine-acetylated derivatives completely blocked cataract development. This was accompanied by significant reductions in protein insolubilization, loss of GSH and epithelial cell apoptosis. The i.p. injected α -crystallin peptides inhibited RGC apoptosis in the I/R model.

Conclusions: Together, our studies suggest that the functional peptides of small heat shock proteins might find use in preventing cataract, and in other eye diseases where apoptosis of retinal cells occur from ischemia, such as in glaucoma and diabetic retinopathy.

COI/Financial disclosure: None

HtrA1 is Required for Retinal Pigment Epithelium Survival During Endoplasmic Reticulum Stress

Sarah Melissa P. Jacobo (Presenter) and Andrius Kazlauskas

Harvard Medical School Department of Ophthalmology, The Schepens Eye Research Institute, 20 Staniford St., Boston, MA USA 02114

Purpose: High Temperature Requirement A1 (HtrA1) is one of the most frequently polymorphic genes in humans with age-related macular degeneration (AMD). Our objective is to understand the role of HtrA1 in the RPE, a site of early and progressive lesions in AMD.

Methods: Cultured ARPE19 cells were stimulated with toxicants and assayed for ER stress markers and HtrA1. To test whether HtrA1 was protective or detrimental to RPE survival during stress, RPE cells where HtrA1 was silenced were treated with ER toxicants. To evaluate the effect of AMD-associated variants (rs1049331 and rs2293870, “dSNP”) of HtrA1 on RPE’s unfolded protein response, we re-expressed WT or dSNP HtrA1 into RPE knockdown cells.

Results: When we induced chronic protein misfolding in RPE cells using tunicamycin, HtrA1 was upregulated just like the canonical chaperones Hsp70 or Hsp90. RPE cells lacking HtrA1 upregulated ER stress markers upon chronic serum deprivation. In response to tunicamycin, HtrA1 knockdown cells failed to resolve ER stress and died, even in the face of upregulated Hsp70/Hsp90 chaperones. Restoration of WT HtrA1 rescued knockdown cells, but dSNP HtrA1 only partially relieved ER stress-associated death. Moreover, even though the CMV-driven re-expression of dSNP exceeded the level of HtrA1 in parental and/or GFP shRNA control cells, dSNP re-expression appeared to lower RPE’s threshold of tolerance for subtoxic doses of tunicamycin.

Conclusions: HtrA1 is part of RPE’s unfolded protein response, and is essential for survival during ER stress. AMD-associated SNPs in HtrA1, rs1049331 and rs2293870, lower RPE’s tolerance for nutrient deprivation, and enhance sensitivity to ER stress.

Novel plate reader-based assay measuring glioprotection in primary optic nerve head astrocytes.

Simon Kaja,¹ Andrew J. Payne,¹ Yuliya Naumchuk,¹ Danish H. Zaidi,¹ Saba Nawazish,¹ Deborah Levy,¹ Jasleen K. Ghuman,¹ Bryan C. Gerdes,¹ Peter Koulen^{1,2}

¹ Vision Research Center, Department of Ophthalmology, and ² Department of Basic Medical Science, University of Missouri – Kansas City, School of Medicine, 2411 Holmes St., Kansas City, MO 64109

Purpose: Drug discovery critically relies on automated cell-based assays. As most assay systems utilize immortalized cell lines, there is an urgent need for primary cell based systems more closely resembling disease physiology.

In glaucoma, optic nerve head astrocytes (ONHAs) undergo significant pathological changes in gene and protein expression, resulting in activation and extracellular matrix remodeling. We developed novel standardized protocols using plate reader-based approaches to measure glioprotection in adult ONHAs.

Methods: We developed 96-well plate-based cell viability assays for primary adult rat ONHAs. Assays determining cellular viability (MTT cell staining), late-stage apoptosis (lactate dehydrogenase [LDH] release), and intracellular redox state (DCFDA staining) were employed to quantify the effect of the prototypic antioxidant Trolox in response to exogenously applied oxidative stress using tert-butylhydroperoxide (tBHP).

Results: Assays measuring cellular viability and damage were successfully implemented for primary ONHAs and plate reader-based technology. The prototypic antioxidant, Trolox, increased the sensitivity of ONHAs to tBHP, shifting the LD50 significantly. LD50 values were $396 \pm 12 \mu\text{M}$ in the LDH release and $383 \pm 3 \mu\text{M}$ in the MTT assay in the presence of $100 \mu\text{M}$ Trolox, compared with $254 \pm 28 \mu\text{M}$ and $191 \pm 5 \mu\text{M}$ for vehicle (0.1% v/v ethanol), respectively.

Conclusions: Primary ONHAs from adult rats can be utilized readily for plate reader-based glioprotection assays. Our data provide important feasibility data for implementation of primary ONHA cell culture for testing novel chemical entities for glaucoma and other disorders affecting the optic nerve and ONH and will allow conversion of these standardized protocols to high-throughput testing strategies.

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Vitamin D, An Improved Therapy for Aberrant Ocular Neovascularisation ?

S. Merrigan¹, O. Galvin¹, A. Reynolds¹ & B. Kennedy¹.

¹ *UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland*

Purpose: The abnormal growth of new, leaky blood vessels underpins the scar formation and blindness in diseases such as Neovascular Age Related Macular Degeneration (nAMD). Currently, 1.8 million Americans have AMD, with an incidence of 2.9 million expected by 2020, of these 10% will progress to nAMD. Existing therapies are not always efficacious and are associated with intraocular injections and high costs. Here, the efficacy and safety of ICCB library hit compound 1 α ,25-Dihydroxyvitamin-D3 and its analogues is being evaluated.

Methods: Compounds were evaluated in zebrafish larvae for ability to inhibit hyaloid (ocular) and intersegmental (non-ocular) developmental angiogenesis. Hits identified were assessed for safety by light microscopy examination of the morphological integrity of larval retinal sections. The efficacy and safety of hits was tested in human retinal pigment epithelial cells, human endothelial cells and mouse aorta. Cell viability (MTT), tubule formation and sprouting angiogenesis (aortic ring assay) in response to treatment was investigated.

Results: 1 α ,25-Dihydroxyvitamin-D3 and several analogues (EB-1089, 22-oxacalcitriol, Calcifediol, Doxercalciferol, Calcipotriol and Tacalcitol) significantly inhibit hyaloid but not intersegmental developmental angiogenesis in larvae. The vitamin D receptor was found to be expressed in larvae at the times of treatment. Overall ocular morphology of drug-treated larvae appears normal. Several drugs cause a dose-dependent decline in cell viability and reduced sprouting angiogenesis in vitro.

Conclusions: Here, we demonstrate that vitamin D significantly and specifically inhibits ocular angiogenesis during zebrafish development. Future experiments will evaluate the molecular mechanisms of anti-angiogenic activity and the efficacy in pre-clinical ocular neovascularisation models.

Sigma 1 Receptor (σ 1R) regulates the oxidative stress response in primary retinal Müller glial cells (1°MGCs) via Nrf2 signaling and system xc⁻, the Na⁺-independent glutamate-cystine exchanger.

Sylvia B. Smith^{1,2,3}, Jing Wang^{1,2}

¹*Department of Cellular Biology and Anatomy, Medical College of Georgia, Georgia Regents University, Augusta, GA*

²*James and Jean Culver Vision Discovery Institute, Georgia Regents University, Augusta, GA*

³*Department of Ophthalmology, Medical College of Georgia, Georgia Regents University, Augusta, GA*

Purpose: Ligands for σ 1R, a unique transmembrane protein whose endogenous physiological function is unknown, have profound retinal neuroprotective properties *in vitro* and *in vivo*. Studies to determine σ 1R retinal neuroprotective mechanisms have focused mainly on neurons, which rely on Müller glial cells (MGCs) for homeostatic support. We asked whether σ 1R mediates retinal oxidative stress responses using MGCs harvested from σ 1R^{-/-} mice.

Methods: WT and σ 1R^{-/-} mouse 1°MGCs were used for (1) ROS determination (CellROX assay, DCFA assay), GSH analysis (CMFDA-staining); (2) qRT-PCR to assess antioxidant genes *SOD1*, *Catalase*, *NQO1*, *HMOX1*, *GST*, *GPX1*; (3) immunoblotting (IB) to detect proteins encoded by these genes; (4) IB and immunofluorescence to analyze Nrf2 and KEAP1, which modulate antioxidant response elements of these genes; (5) uptake of ³H-glutamate via system xc⁻ (needed for GSH synthesis); (6) qRT-PCR/IB analysis of xCT, the unique component of system xc⁻.

Results: Compared to WT-1°MGCs, endogenous ROS increased significantly; *SOD1*, *Catalase*, *NQO1*, *HMOX1*, *GST*, *GPX1* expression decreased; SOD1, catalase, NQO1, GPX1 protein levels decreased significantly in σ 1R^{-/-} 1°MGCs. Nrf2 gene/protein expression decreased and KEAP1 levels increased significantly in σ 1R^{-/-} 1°MGCs. Nrf2-ARE binding affinity decreased markedly in σ 1R^{-/-} 1°MGCs. xCT expression decreased markedly in σ 1R^{-/-} 1°MGCs; uptake of ³H-glutamate was ~50% less than WT cells. GSH and GSH/GSSG ratios decreased in σ 1R^{-/-} 1°MGCs.

Conclusions: MGCs lacking σ 1R manifest alterations of oxidative stress indicators, perturbation of antioxidant balance, suppression of Nrf2 signaling, impaired function of system xc⁻. Retinal neuroprotective roles of σ 1R may be linked directly to oxidative stress-mediating properties of supportive glial cells.

Familial Alzheimer's disease mutations impair retinal sensitivity and can be protected by methionine restriction.

Vasudevaraju Padmaraju¹, Anis Ahmad^{1,2}, Panneerselvam Chinnakkannu¹, Zsolt Ablonczy², Craig E. Crosson², Kumar Sambamurti^{1,2}

¹*Department of Neuroscience, Medical University of South Carolina, Charleston, USA*

²*Department of Ophthalmology, Medical University of South Carolina, Charleston, USA*

Purpose: Amyloid beta (A β) protein is implicated in Alzheimer's disease (AD) and age-related macular degeneration (AMD) pathogenesis. A β precursor (APP) and its metabolites are present in the retina and ocular fluids. Failure of γ -secretase (GS), the A β generating enzyme, leads to impairment of retinal pigment epithelium (RPE) barrier function and paradoxically increases A β at low doses of GS inhibitor (GSI). Herein we are reporting that familial AD (FAD) mutant GS impairs retinal function and that we can provide partial protection by dietary restriction.

Methods: APP metabolite levels were determined in methionine-restricted APP695 transfected cell cultures and AD mouse models. AD transgenic mice were treated with GSI or methionine restricted diet and analyzed by electroretinography (ERG).

Results: Transgenic mice showed impairment in A and B waves. Acute GSI treatment impaired both A and B waves. Methionine restricted mice show significant increases in A-wave amplitude in 6 and 9 month treated mice but B wave did not change. Biochemical analysis show that soluble A β decreased in the brain of mice treated for 6 months but the difference was not maintained after 16 months of treatment.

Conclusion: These data provide evidence that mild chronic impairment of γ -secretase results in impairment of retinal function. Similarly acute inhibition of γ -secretase also impairs retinal sensitivity. The chronic impairment is partially prevented by methionine restriction suggesting that this paradigm may help in preventing age-related protein accumulation in diseases such as AMD. Additional studies are required to both understand the mechanisms of protection and optimization of dietary intervention.

Screening pharmacological compounds using an *in vitro* model of proliferative vitreoretinopathy

Whitney A. Greene¹, Elaine D. Por¹, Anthony Johnson¹, Heuy-Ching Hetty Wang¹

¹United States Army Institute of Surgical Research, Ocular Trauma, Fort Sam Houston, San Antonio TX, USA

Purpose: To analyze the ability of pharmacological compounds to prevent migration, proliferation, and contraction of the retinal pigment epithelium. RPE cells derived from induced pluripotent stem cells (iPS-RPE) exhibit the morphology and function of *in vivo* RPE. These cells were used to develop a highly relevant *in vitro* model that allows investigation of the cellular mechanisms that underlie the three phases of PVR pathology: migration, proliferation, and contraction.

Methods: iPS-RPE was grown to confluency on fibronectin-coated transwells. The cell monolayer was scratched to create a wound. After wounding, cells were treated with 5% vitreous fluid, plus pharmacological compounds to inhibit histone deacetylase (HDAC) /histone acetyl transferase (HAT), Rho GTPase, TGF β and PDGFR pathways. At 21 days after wounding, the iPS-RPE was labeled by immunofluorescence for α -smooth muscle actin and β -catenin. Western blot analysis was performed to detect proteins that regulate epithelial-mesenchymal transition.

Results: Treatment of iPS-RPE with 5% vitreous after wounding induced profound contraction of the wound area and significant increase in expression of smooth muscle actin. Treatment with trichostatin A to inhibit HDAC function resulted in decreased smooth muscle actin expression. Inhibition of Rho GTPase activity with ROCK inhibitor reduced contraction of the wound area and decreased smooth muscle actin. Inhibition of TGF β and PDGFR signaling did not prevent wound contraction or smooth muscle actin expression.

Conclusions: These results indicate that iPS-RPE provide a physiologically relevant model that can be used to screen pharmacological compounds for the ability to prevent migration, proliferation, and contraction of the RPE.

cGMP/PKG Signaling Suppresses Inositol 1,4,5-Trisphosphate Receptor Phosphorylation and Promotes Endoplasmic Reticulum Stress in Photoreceptors of CNG Channel-deficient Mice

Xi-Qin Ding¹, Hongwei Ma¹, Michael Butler¹, Arjun Thapa¹, Wolfgang Baehr², Mirja Koch³, Martin Biel³, and Stylianos Michalakis³

¹The Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA

²The John A. Moran Eye Center, University of Utah, Salt Lake City, Utah, USA

³Center for Integrated Protein Science Munich (CIPSM) and Department of Pharmacy - Center for Drug Research, Ludwig-Maximilians-Universität München, Munich, Germany

Purpose: Photoreceptor cyclic nucleotide-gated (CNG) channels play a pivotal role in phototransduction. Mutations in the cone CNG channel subunits CNGA3 and CNGB3 are associated with achromatopsia and cone dystrophies. We have shown endoplasmic reticulum (ER) stress-associated apoptotic cone death and increased phosphorylation of the ER calcium channel inositol 1,4,5-trisphosphate receptor (IP₃R) in CNG channel deficient mice. We also showed a remarkable elevation of the cellular cyclic guanosine monophosphate (cGMP) level and an increased activity of the cGMP-dependent protein kinase (protein kinase G, PKG) in CNG channel-deficient retinas. This work investigated whether cGMP/PKG signaling regulates ER stress and IP₃R activity in CNG channel deficiency.

Methods: Two cone CNG channel-deficient mouse lines, *Cnga3*^{-/-}/*Nrl*^{-/-} (*Cnga3* deficiency on a cone-dominant background) and *Cnga3*^{-/-}/*Nrl*^{-/-}/*Gucy2e*^{-/-}, were used in this study. Inhibition of cGMP/PKG signaling was achieved by using PKG inhibitors KT5823 and Rp-8-Br-cGMPS, and by deleting *Gucy2e*, the gene encoding retinal guanylate cyclase-1. Retinal cGMP level and PKG activity were analyzed by ELISA and colorimetric analysis, respectively. TUNEL labeling was performed to assess cone death, glial fibrillary acid protein (GFAP) labeling was performed to evaluate Müller glial cell activation, and levels of phospho-eIF2 α and phospho-IP₃R were analyzed to evaluate ER stress and IP₃R activity, respectively.

Results: We found that treatment with PKG inhibitor or deletion of *Gucy2e* effectively reduced PKG activity and apoptotic cone death, increased expression levels of cone proteins, and decreased activation of Müller glial cells in CNG channel-deficient mice. Furthermore, inhibition of PKG activity and deletion of *Gucy2e* significantly reduced ER stress, manifested as reduced levels of phospho-eIF2 α , and increased phosphorylation of IP₃R.

Conclusion: This work demonstrates a role of cGMP/PKG signaling in ER stress and ER calcium channel regulation, and provides insights into the mechanism of cone degeneration in CNG channel deficiency.

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Chronic tadalafil treatment attenuates increases in IOP and retinopathy in sGC α_1 ^{-/-} mice

Ana C Dordea, Sara Vandenwijngaert, Robert E Tainsh, Kaitlin Allen, Wolfgang S Lieb, Emmanuel S Buys

¹Department of Ophthalmology & Visual Sciences, Case Western Reserve University, Cleveland, OH and ²Department of Ophthalmology, University of Colorado, Aurora, CO

Purpose: Primary open angle glaucoma (POAG) is a progressive eye disease characterized by increased intraocular pressure (IOP) and loss of retinal ganglion cells (RGC). The nitric oxide (NO)-soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP) pathway has been associated with POAG: genetic and epidemiological studies implicated impaired NO-cGMP as a possible pathogenic mechanism in POAG, and preclinical studies demonstrated the ability of NO-donor compounds (e.g. latanoprostene bunod) to lower IOP. Also, mice deficient in sGC α_1 (sGC α_1 ^{-/-}) develop increased IOP and retinal neurodegeneration. We hypothesized that enhancing cGMP signaling with the phosphodiesterase 5 inhibitor tadalafil (Cialis), would attenuate the development of glaucoma in sGC α_1 ^{-/-} mice.

Methods: In a pilot study, female sGC α_1 ^{-/-} mice were fed a tadalafil-containing or control diet (n=7 and n=8, respectively) for eight months. IOP was measured bimonthly under isoflurane anesthesia. At the end of the study, eyes were harvested and RGCs were stained with β III-tubulin for density assessment.

Results: The IOP increase typically observed in sGC α_1 ^{-/-} mice was attenuated in tadalafil-fed mice: over an eight month period, IOP increased 2 $\pm$ 1 mmHg in tadalafil-fed compared to 5 $\pm$ 1 mmHg in control mice (P<0.01). Additionally, RGC counts were higher in 12-month old tadalafil-fed sGC α_1 ^{-/-} mice than age-matched control-fed mice (51 $\pm$ 1 vs. 48 $\pm$ 1 counts/mm², respectively, P<0.05).

Conclusion: The use of tadalafil attenuated the increase in IOP and neurodegeneration in sGC α_1 ^{-/-} mice. Tadalafil may therefore serve as a potential therapeutic in glaucoma, particularly in patients presenting with dysfunctional NO-sGC signaling.

Sigma1 Receptor-Induced Correlates Between Caspase Activity and Mitochondria Membrane Potential in Glucose and Oxygen Deprived Retinal Ganglion Cells.

Dorette Z. Ellis^{1,2}, Linya Li², Yong Park², Brett Mueller², Hai-Ying Ma² and Thomas Yorio^{2,3}

¹*Department of Pharmaceutical Sciences*

²*North Texas Eye Research Institute*

³*Pharmacology & Neuroscience University of North Texas Health Science Center, Fort Worth, Texas*

Purpose: Previous studies showed that activation of the sigma1 receptor (σ -1r) protected retinal ganglion cells (RGC) from death in an oxygen and glucose deprived (OGD) model for oxidative stress. In these studies we examined the mechanisms by which σ -1r protects RGCs from death. Specifically, we examined the role the σ -1r in regulating mitochondrial function and caspase activity.

Methods: RGCs were isolated from rat pups and subjected to OGD in the presence or absence of σ -1r agonist and antagonist and AAV- σ -1r vector, used to increase σ -1r expression. Mitochondrial membrane potential was measured using JC1 dye. Caspase 3 and 7 activities were measured using luminescent assay kit.

Results: OGD in RGCs resulted in decreased mitochondrial membrane potential when compared to normoxic RGCs. Addition of σ -1r agonists restored the mitochondrial membrane potential comparable to normoxic conditions while σ -1r antagonists abolished these effects. Overexpression of the σ -1r resulted in the restoration of RGCs' health following OGD deprivation. Caspase activity was increased in response to ODG and decreased by σ -1r agonist, pentazocine and σ -1r overexpression. Furthermore, there was a time course correlate between σ -1r-induced changes in mitochondrial membrane potential and caspase activity.

Conclusions: These data suggest that σ -1r restores RGCs function following OGD; particularly mitochondrial function which is vital to the health of the cells.

COI/Financial Disclosure: None

Neuroprotective effects of transcription factor Brn3b in an ocular hypertension rat model of glaucoma

Dorota L. Stankowska¹, (Presenter), Raghu R. Krishnamoorthy¹

¹*University of North Texas Health Science Center, Department of Cell Biology and Immunology, North Texas Eye Research Institute, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107*

Purpose: Glaucoma is an optic neuropathy commonly associated with elevated intraocular pressure (IOP), leading to optic nerve head (ONH) cupping, axon loss and apoptosis of retinal ganglion cells (RGCs) which could ultimately result in blindness. Brn3b is a class-4 POU domain transcription factor that plays a key role in RGC development, axon outgrowth and pathfinding. Previous studies suggest that a decrease in Brn3b levels occurs in animal models of glaucoma. The goal of this study was to determine if adeno-associated virus (AAV)-directed overexpression of the Brn3b protein could have neuroprotective effects following elevated IOP-mediated neurodegeneration.

Methods: IOP was elevated in one eye of Brown Norway rats (*Rattus norvegicus*), following which the IOP-elevated eyes were intravitreally injected with AAV constructs encoding either the GFP (rAAV-CMV-GFP and rAAV-hsyn-GFP) or Brn3b (rAAV-CMV-Brn3b and rAAV-hsyn-Brn3b). Retina sections through the ONH were stained for synaptic plasticity markers and neuroprotection was assessed by RGC counts and visual acuity tests.

Results: AAV-mediated expression of the Brn3b protein in IOP-elevated rat eyes promoted an upregulation of growth associated protein-43 (GAP-43), actin binding LIM protein (abLIM) and acetylated α -tubulin (ac-Tuba) both posterior to the ONH and in RGCs. An increase in Cholera toxin subunit B labeling along the optic nerve axons was found in IOP-elevated rAAV-hsyn-Brn3b injected rat eyes compared to IOP-elevated rAAV-hsyn-GFP injected rat eyes. Additionally, intravitreal rAAV-hsyn-Brn3b administration significantly restored the visual optomotor response in IOP-elevated rat eyes.

Conclusion: AAV-mediated Brn3b protein expression may be a suitable approach for promoting neuroprotection in animal models of glaucoma.

Receptor protein tyrosine phosphatase sigma (RPTP- σ) increases pro-MMP activity in oxidatively stressed trabecular meshwork cell line

Elie Beit-Yannai¹, Michal Zaiden¹

¹Clinical Biochemistry and Pharmacology department, Ben-Gurion university of the Negev, Beer-Sheva, Israel

Purpose: To explore the role of phosphatases in the eye drainage system, by over expression of the RPTP- σ in a normal human trabecular meshwork (NTM) cell line.

Methods: Following transfection with RPTP- σ , efficacy, expression and location were evaluated by flow cytometry, WB immunocytochemistry and confocal microscopy. Transfected NTM (NTM^T) cells were analyzed for cell viability by MTT, for metalloproteinase (MMP) activity by zymography, and for phosphatase activity following oxidative stress by the DiFMUP method. Assays were carried out in the presence and absence of a specific RPTP- σ inhibitor (PTP-IV).

Results: Transfection efficacy measured during GFP expression at 48 h revealed that GFP expression was significantly higher in NTM^T cells. WB analysis showed a significant increase in RPTP- σ expression in NTM^T cells. No significant differences in cell vitality were found between NTM^T and control cells after oxidative stress. ProMMP-2 and proMMP-9 showed significantly higher activity in NTM^T cells. Serine/threonine phosphatase activity after oxidative stress was significantly higher in NTM^T cells vs. controls. PTP-IV presence caused 15% inhibition of RPTP- σ expression in NTM cells and a significant inhibition in NTM^T. NTM^T exposed to PTP-IV, the activities of the pro forms of MMP-9 and MMP-2 and of the active form of MMP-9 were significantly inhibited found.

Conclusions: According to our results, RPTP- σ is constitutively expressed in NTM cells. Our findings suggest that oxidative stress causes the general phosphatase balance in NTM cells to change. Our results also showed that RPTP- σ expression levels affect the activity of different metalloproteinase (MMP) forms.

The Ago-PAM GAT211 Decreases Retinal Ganglion Cell Loss in the Nee Mouse Model of Ocular Hypertension

Elizabeth A. Cairns¹, Alex J. Straiker², Pushkar M. Kulkarni³, Ganesh A. Thakur³, William H. Badridge^{4,5}, and Melanie E.M. Kelly^{1,5}

¹Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada

²Department of Psychological and Brain Sciences, Indiana University, Bloomington, Indiana, United States of America

³Department of Pharmaceutical Sciences & Centre for Drug Discovery, Northeastern University, Boston, Massachusetts, United States of America

⁴Department of Medical Neuroscience, Dalhousie University, Halifax, Nova Scotia, Canada

⁵Department of Ophthalmology and Visual Sciences, Dalhousie University, Halifax, Nova Scotia, Canada

Purpose: Glaucoma is a blinding eye disease involving loss of retinal ganglion cells (RGCs). The most important modifiable risk factor in glaucoma is intraocular pressure (IOP) which is the current target for glaucoma therapy. However, additional therapies directed at RGC neuroprotection in addition to IOP may be beneficial. Activation of cannabinoid receptor 1 (CB1) in experimental models of glaucoma reduces IOP and decreases RGC loss. Positive allosteric modulator (PAMs) of CB1 may provide another mechanism to both lower IOP and decrease RGC loss, while having a reduced potential for negative side effects. Our experiments examined the effects of GAT211, a novel CB1 ago-PAM on IOP and cell loss in the nee mouse, an experimental model of ocular hypertension (OH).

Methods: Nee mice received either 5 μ L of 5mM GAT211 or vehicle topically, once per day for 12 days. On the final day, IOP was measured using rebound tonometry 12 hours after application of either GAT211 or vehicle. Mice were then sacrificed, and retinas were assessed for cell loss using the immunohistochemical marker Brn3a.

Results: Nee mice eyes receiving GAT211 for 12 days had an average IOP of 21.2 ± 1.9 mmHg, compared with 24.3 ± 1.7 mmHg in vehicle eyes ($p < 0.05$, $n = 10$, paired t-test). Nee mice eyes receiving GAT211 had an average RGC density of 1692 ± 187 cells/mm², compared with 885 ± 370 cells/mm² in vehicle-treated eyes ($p < 0.05$, $n = 4$, paired t-test).

Conclusions: The novel CB1 ago-PAM GAT211 lowers IOP and reduces RGC loss in a genetic model of OH.

Recovery profile of human trabecular meshwork cells following withdrawal from prolonged corticosteroid treatment

Guorong Li, W. Michael Dismuke, Iris Navarro, Kristin M Perkumas, *David F Woodward, W. Daniel Stamer.

Ophthalmology Department, Duke University, Durham, NC

**Department of Biological Sciences, Allergan, Inc., Irvine, California, United States*

Purpose: Corticosteroid-induced ocular hypertension can be reproduced in mice, which display all four hallmarks of the human disease. Interestingly, upon removal of corticosteroids, intraocular pressure returns to normal within a week. The purpose of this study was to examine the biochemical changes that occur in human trabecular meshwork (HTM) cells over time following withdrawal from prolonged corticosteroid treatment.

Methods: HTM cells (HTM120, HTM123 and HTM124) from three individual donors were cultured and treated with dexamethasone (Dex) 100 nM in 1% FBS supplemented medium for 1 or 4 weeks. The cell culture supernatant was collected 3 times/week. The secretion of myocilin (MYOC), matrix metalloproteinase-2 (MMP2) and fibronectin (FN) were analyzed by Western Blot.

Results: MMP2 protein levels were reduced ~40% by Dex treatment ($p < 0.01$). MMP2 returned to normal levels following Dex withdrawal within a week, even after four weeks of treatment. In contrast, a significant increase in MYOC levels (up to 600%) remained elevated two weeks after Dex withdrawal, even after only one week of treatment. Interestingly, FN showed a delayed response to one week of treatment, not reaching significantly higher levels until one week after Dex withdrawal. However, a significant ~183% increase in FN was observed following four weeks of Dex, not returning to normal levels until four weeks post withdrawal.

Conclusions: Results suggests that the rapid return of IOP following cessation of corticosteroid treatment may be due to recovery of normal MMP secretory function by HTM cells and homeostatic extracellular matrix degradation.

Effects of topical baicalein on intraocular pressure in rodents

Hoi-lam Li¹, Ho-lung Chan¹, Chi-ho To¹, Chi-wai Do¹

¹*School of Optometry, The Hong Kong*

Purpose: Baicalein (5,6,7-trihydroxyflavone) is a natural flavonoid derived from the root of *Scutellaria baicalensis*. It is frequently found in vegetables and is a common traditional Chinese medicine. We have previously demonstrated that intraperitoneal administration of baicalein lowers intraocular pressure (IOP) in rats. In this study, we investigated whether similar ocular hypotensive effects were observed after topical application rather than intraperitoneal injection.

Methods: Sprague-Dawley (SD) rat and C57BL/6J (B6) mice (2-4 months old) were used. IOP was measured by rebound tonometry under awake condition. Topical baicalein (20 μ l, 10mM) was applied to the treatment eye twice separated by 10-min intervals while phosphate buffered saline (PBS) was used in the fellow eye as a control. IOP measurements were conducted before and after drug administration (1.5, 3, 6, 24, 48 and 72h) in both light and dark phases.

Results: Topical administration of baicalein caused significant ocular hypotensive responses within 6 hours after drug treatment in both species. In SD rats, the maximum IOP reduction was 0.79 (n=23, p<0.01) and 2.14 (n=23, p<0.01) mmHg under light and dark phases, respectively. In B6 mice, the maximum ocular hypotensive effect was 1.44 (n=23, p<0.01) and 2.16 (n=23, p<0.01) mmHg under light and dark conditions, respectively. No significant IOP-lowering effect was observed 24 to 72 hours after treatment.

Conclusions: Topical application of baicalein acted across species and triggered a transient IOP reduction in rodents. However, its ocular hypotensive effect was smaller than that of intraperitoneal injection. Further work is required to improve and enhance drug permeability across cornea.

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Functional characterization of LOXL1 regulatory variants in pseudoexfoliation glaucoma

Inas F. Aboobakar¹, Yutao Liu^{2,3}, Shiro Miura⁴, Joshua Wheeler², Xue-Jun Qin², Benjamin T. Whigham², Allison Ashley-Koch², Michael A. Hauser^{2,3}, and R. Rand Allingham³

¹ *Duke University School of Medicine, Durham, NC*

² *Department of Medicine, Duke University Medical Center, Durham, NC*

³ *Department of Ophthalmology, Duke University Medical Center, Durham, NC*

⁴ *Department of Medicine, Kurume University School of Medicine, Kurume City, Japan*

Purpose: Variants in the LOXL1 gene are associated with risk for pseudoexfoliation glaucoma (XFG) in populations worldwide, but their role in disease pathogenesis remains unknown. In a South African XFG dataset, the most strongly associated variants are located in intron 1 of LOXL1, which lies upstream of the regulatory antisense RNA for LOXL1 (LOXL1-AS1). Here, we evaluate whether these intronic variants alter LOXL1-AS1 promoter activity.

Methods: Using a dual luciferase reporter assay, we tested the effects of 3 single nucleotide polymorphisms (rs1550437, rs6495085, and rs6495086), all of which are located in LOXL1 intron 1 and highly associated with XFG. The SNP all-risk and all-protective haplotypes were cloned and promoter activity was tested in HEK293 (embryonic kidney) and B3 (lens epithelium) cell lines. We also evaluated expression of LOXL1-AS1 in ocular tissues and in a cDNA panel containing multiple systemic tissues.

Results: Data from the dual-luciferase reporter assay indicates that intron 1 of LOXL1 contains a promoter. The haplotype of three risk alleles had 39% higher promoter activity in the HEK293 cell line ($p=4.5 \times 10^{-7}$) and 79% higher activity in the B3 cell line ($p=4.3 \times 10^{-8}$) compared to the non-risk haplotype. A novel LOXL1-AS1 splice variant was identified in multiple ocular and systemic tissues.

Conclusions: Our data suggests that XFG-associated variants contribute to disease risk by increasing LOXL1-AS1 promoter activity, which may decrease LOXL1 expression. Improved understanding of the regulation of LOXL1 and LOXL1-AS1 in the XFG disease process will likely enable development of effective therapeutic strategies for this common, potentially blinding disorder.

Stimulation of TLR3 leads to lysosomal exocytosis and ATP release from both RPE cells and optic nerve head astrocytes

Jason C. Lim¹, Wennan Lu¹, Nestor Mas Gomez¹, Alan M. Laties², Claire H. Mitchell^{1,3}

Departments of ¹Anatomy and Cell Biology, ²Ophthalmology, and ³Physiology, University of Pennsylvania, Philadelphia, PA 19104.

Purpose: The release of transmitter by non-neural cells occurs via both vesicular and non-vesicular pathways. In this study, we asked whether release of the transmitter ATP accompanies lysosomal exocytosis and examined triggers for release.

Methods: Mouse RPE cells, ARPE-19 cells, and rat optic nerve head astrocytes were used for experiments. The luciferin assay was used to detect ATP release, while lysosomal exocytosis was detected with an acid phosphatase assay.

Results: ATP was released from both optic nerve head astrocytes and RPE cells following stimulation of TLR3 with agonist poly(I:C). This ATP release was blocked by vesicular transport inhibitor NEM, but not pannexin channel inhibitor carbenoxelone. Poly(I:C) increased extracellular levels of lysosomal enzyme acid phosphatase along with the ATP, implying ATP release via lysosomal exocytosis. Fluorescent MANT-ATP showed particulate staining which colocalized with lysosomal stains, suggesting lysosomal storage of ATP. The time course of release differed between the cells, with extracellular ATP levels peaking 15-30 min after poly(I:C) addition in astrocytes, but over an hour in RPE cells. Starvation, but not rapamycin, prevented the release from both cell types, consistent with the specific depletion of ATP stored in lysosomes upon starvation. Both ATP and acid phosphatase were released upon stimulation of cells with scrambled siRNA, suggesting this was a general response to TLR3 stimulation that may have broad implications.

Conclusion: Lysosomal exocytosis leads to the release of ATP from both astrocytes and RPE cells following stimulation of TLR3. This identifies a new pathway by which purinergic signaling can be activated.

Acid Sphingomyelinase Plays a Role in Ischemia-induced Retinal Degeneration

Jie Fan and Craig E Crosson.

Purpose: Retinal ischemia is a common cause of visual impairment and blindness. Acid sphingomyelinase (ASM) is an enzyme that catalyzes the hydrolysis of sphingomyelin to ceramide, a key mediator of cell-stress responses and cell death. The purpose for this study is to investigate whether ASM plays a critical role in retinal ischemic injury.

Methods: ASM^{+/-} mice and wild-type (WT) littermates were evaluated for changes in ASM activity, retinal function and morphology following retinal ischemia for 45min.

Results: In WT mice, retinal ischemia significantly decreased a- and b-wave amplitudes by 43 and 82%, respectively. In ASM^{+/-} mice, retinal ischemia also decreased mean a- and b-wave amplitudes by 28 and 67%; respectively; however, these declines were significantly less than that observed in WT mice. Morphometric analysis of ischemic eyes from WT demonstrated significant decreases in INL and IPL thickness of 43 and 77%, respectively, and a 65% decrease in cell bodies in the GCL. The ischemic eyes from ASM^{+/-} mice revealed a decrease in INL and IPL thickness of 22 and 58%, respectively; but this decrease was significantly less than that observed in WT. In addition, significantly more surviving cell bodies in GCL were observed in ischemic eyes from ASM^{+/-} compared to WT mice.

Conclusion: Our results demonstrate that the reduction in ASM activity/expression provides partial protection from ischemic injury. Hence, the production of ceramide and subsequent mediators likely play important roles in ischemic retinal injury. Inhibition of ASM may present new opportunities for the treatment of retinal ischemic disorders.

INVESTIGATION OF LENTIVIRAL VECTORS AS ANTERIOR SEGMENT GENE TRANSDUCING AGENTS IN THE MOUSE

J. Cameron Millar^{1,2}, Margaret Holder¹, Abbot F. Clark^{1,2}

¹*Department of Cell Biology & Immunology*

²*North Texas Eye Research Institute, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107 USA*

Purpose: We investigated the suitability of lentiviral vectors for transduction of the murine anterior segment. We injected copGFP HIV and FIV vectors into mouse eyes and studied expression of fluorescence and immunogenicity.

Methods: Naïve BALB/cJ and C3H/HeJ mice, 8-9 weeks (♀) were studied. Animals in each strain were divided into 2 cohorts (N=5/cohort). One cohort of BALB/cJ and C3H/HeJ animals received (OS only) an intravitreal (IVT) injection (1.336×10^6 ifu/2 μ L injection bolus) of FIV vector (pSIF1-H1-siLuc-copGFP). The other cohort of BALB/cJ and C3H/HeJ animals received (OS only) an IVT injection of HIV vector (pSIH1-H1-siLuc-copGFP (8.7×10^6 ifu/2 μ L injection bolus)). From Day = 6 following injection, eyes were examined twice/week for fluorescence, and by ophthalmoscopy. On Day = 40, animals were sacrificed and eyes processed for histology.

Results: Lentivirus-injected eyes in C3H/HeJ animals showed significant fluorescence in the chamber angle, starting at Day = 9 (HIV) and Day = 20 (FIV), which persisted to sacrifice. FIV-injected eyes in BALB/cJ animals showed marginal fluorescence, starting at Day = 9. HIV-injected eyes in BALB/cJ animals showed no fluorescence. No immunogenicity was seen. In both HIV and FIV-injected C3H/HeJ eye sections, fluorescence was seen in the trabecular meshwork (TM). In FIV-injected BALB/cJ eyes, marginal fluorescence was seen in the TM. Injected eyes also showed marginal fluorescence in retina and lens. In HIV-injected BALB/cJ eyes, no fluorescence was seen. No evidence of inflammatory infiltrates was seen.

Conclusions: Lentiviral vectors are able to yield sustained transgene expression in C3H/HeJ mice TM following IVT injection, without promoting immunogenicity.

NO and COX products regulate the retinal vessel diameters in diabetic patients.

Line Petersen, Toke Bek

Department of Ophthalmology, Aarhus University Hospital, Denmark.

Purpose: Diabetic retinopathy is characterized by hypoxia in the retinal periphery and accompanying dilatation of the larger vessels in the central retina. Previous studies have shown that nitric oxide (NO) and cyclooxygenase (COX) products mediate hypoxia-induced vasodilatation under normal conditions *in vitro* and *in vivo*. The purpose of the present study was to examine whether this could be reproduced in diabetic patients and thereby point to new treatment strategies for vascular changes in diabetic retinopathy.

Methods: Eighteen diabetic patients (20-30 years) were examined using the Dynamic Vessel Analyzer (DVA) with diameter measurements of a retinal arteriole and venule during rest, during isometric exercise and during flicker stimulation. The vessel diameters were studied before and during breathing a hypoxic gas mixture before and during intravenous infusion with the NOS inhibitor L-NMMA. All examinations were repeated on a second day after topical administration of the COX-inhibitor diclofenac.

Results: The resting diameters of the arterioles and venules increased significantly during hypoxia ($p < 0.0001$) which was reversed by the infusion of L-NMMA. The arteriolar contraction during isometric exercise decreased significantly during hypoxia and L-NMMA infusion alone ($p = 0.016$) and was non-significantly increased by diclofenac, whereas the contraction of the venules increased significantly during hypoxia and L-NMMA infusion together ($p = 0.001$). Flicker-induced dilatation of arterioles and venules were significantly reduced during hypoxia ($p < 0.0001$).

Conclusions: NO and COX products are involved in the regulation of retinal vessel diameter in diabetic patients. These mechanisms may be used as targets for normalizing pathologic diameter changes of retinal vessels in diabetic retinopathy.

Sphingolipid Signaling – New Insights into the Mechanism of Ganglion cell Death and Glaucoma

Madeline L. Budda^{1,2,3}, Joseph Wilkerson^{1,2,3}, Megan Stiles^{2,3}, Tuan-Phat Huynh^{2,3}, William Johnson^{2,3}, Nawajes Mandal^{1,2,3}

¹Cell Biology, ²Ophthalmology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA.

³Dean McGee Eye Institute, Oklahoma City, OK, USA

Purpose: Sphingolipids are essential for development and maintenance of neural tissues. Many sphingolipid metabolites, such as ceramide, sphingosine and sphingosine-1-phosphate are bioactive lipids that act as second messengers to regulate cellular functions including apoptosis and inflammation. Acid Ceramidase (ASAH1) is a critical enzyme which converts ceramide to sphingosine. Little is currently known about the role of ASAH1 in retinal neurons. Retinal neuronal cell death is the ultimate cause of blindness in many diseases including glaucoma. This study characterized a novel mouse line containing a retina-specific deletion of *Asah1* gene, which induces progressive retina ganglion cell death.

Methods: The mouse line was characterized using electroretinography, fundus photography, fluorescein angiography, ocular coherence tomography, tonometry, and histological analyses. Phenotypic abnormalities were validated using immunohistochemistry. Relative levels of ceramide, sphingosine, and sphingosine-1-phosphate were determined using mass spectrophotometry.

Results: The ganglion cell layer was predominately affected. Ganglion cells were significantly reduced with concomitant reduction in inner retinal thickness. Knocking down of ASAH1 in the retina developed a fundus white spots with aging, which could be related to the loss of ganglion cells.

Conclusions: The novel mouse model indicated a potential link between the bioactive sphingolipids and preservation of the retinal ganglion cells. The accumulation of ceramide in the retina might have induced apoptotic ganglion cell death; or microglial activation, inflammation that followed by cell death. This animal model could serve as a model for human primary open angle glaucoma (POAG) to elucidate the mechanism of ganglion cell death, and to evaluate novel therapeutics for glaucoma.

Shear Stress Regulation of eNOS Promoter Activity in Schlemm's Canal Cells

Nicole E. Ashpole¹, Kristin Perkumas², W. Daniel Stamer^{1,2}

¹*Biomedical Engineering, Duke University, Durham, NC*

²*Duke Eye Center, Duke University, Durham, NC*

Purpose: Nitric oxide (NO) is a molecule that increases conventional outflow facility and lowers IOP. It appears that a major contributor to the IOP-lowering effects of NO is the shear stress-regulated enzyme, endothelial nitric oxide synthase (eNOS) in Schlemm's canal (SC). To examine the eNOS promoter activity in SC, we constructed two reporter constructs, eNOS-GFP (green fluorescent protein) and eNOS-SEAP (secretory alkaline phosphatase) adenoviruses and tested them in human SC monolayers exposed to shear stress.

Methods: SC cells were seeded into Ibidi flow chambers and allowed to mature before adenovirus transduction with – eNOS-GFP, eNOS-SEAP or control (ubiquitin-GFP). After two days, cells were exposed to 0.1 or 10 dynes/cm² of continuous shear for 6, 12 or 24 hours. GFP expression was assessed by confocal microscopy and Western Blot. SEAP was quantified from conditioned media with a SEAP Reporter assay. HUVECs (human umbilical vein endothelial cells) were tested for comparisons.

Results: Both HUVECs and SC cells transduced with eNOS-GFP, displayed amplified GFP fluorescence at 10 dynes/cm² compared to 0.1 dynes/cm², with the largest increase occurring at 24 hours. GFP protein in cell lysates displayed a similar pattern (~2-fold increase). Shear stress also induced GFP expression in cells transduced with ubiquitin-GFP, which contains a single consensus shear-stress response element. The eNOS-SEAP adenovirus experiments had the greatest shear-driven increase in expression (4000-fold).

Conclusions: Results suggest that the eNOS promoter in SC cells is shear responsive, and reporter constructs can be used as a sensitive tool to estimate and localize shear forces in SC in situ and in vivo.

Involvement of the Endothelin Receptor A in a Rat Model of Ocular Hypertension

Nolan R. McGrady^{1,2}, Alena Z. Minton^{1,2}, Raghu R. Krishnamoorthy^{1,2}

¹Cell Biology and Immunology, UNT Health Science Center, Fort Worth, TX

²North Texas Eye Research Institute, Fort Worth, TX

Purpose: The endothelin system of peptides and their receptors have been implicated for their neurodegenerative role in glaucoma. The purpose of this study was to determine the involvement of the ET_A receptor in the retina following Morrison's model of glaucoma in rats.

Methods: IOP was elevated in the left eye of adult male retired breeder Brown Norway rats using a modified Morrison's model of glaucoma (by injection of hypertonic saline through episcleral veins) while the contralateral eye served as the control. The rats were maintained for either two or four weeks following IOP elevation and sacrificed. Retinal sections were obtained from both control and IOP-elevated eyes, and analyzed for changes in ET_A receptor expression using immunohistochemistry. ET_A receptor immunostaining was co-localized with β -III-Tubulin, which is selectively expressed in retinal ganglion cells.

Results: After two weeks of IOP elevation an increase in immunostaining for ET_A receptors was observed in multiple retinal layers including the outer and inner plexiform layers with a modest increase in the GCL. Following four weeks of IOP elevation, ET_A receptor expression was modestly increased in the outer plexiform layer and GCL of the retina, compared to the corresponding contralateral eyes.

Conclusion: Elevated intraocular pressure results in a time-dependent change in ET_A receptor expression. Results suggest changes in ET_A receptor expression could be associated with neurodegenerative changes in glaucoma.

Effects of Rho kinase inhibitor AR-13324 on the actin cytoskeleton and on TGF β 2- and CTGF-induced fibrogenic activity in Human Trabecular Meshwork Cells.

Padmanabhan P Pattabiraman¹, Ponugoti Vasanth Rao^{1,2}, Cheng-Wen Lin³ and Casey Kopczynski³

¹Ophthalmology, ²Ophthalmology and Pharmacology and Cancer Biology, ¹Duke University Medical Center, Durham, North Carolina

³Aerie Pharmaceuticals, Research Triangle Park, NC

Purpose: To test the effects of Rho-associated protein kinase/norepinephrine transporter (ROCK/NET) inhibitor AR-13324 on actin cytoskeleton and TGF β 2- and CTGF-induced expression of fibrogenic and myofibroblast markers in primary Human Trabecular Meshwork (HTM) cells. Trabecular meshwork fibrosis is correlated with elevated intraocular pressure in patients with glaucoma.

Methods: HTM cells were treated for 4h with AR-13324 (0.25, 0.5, 5, and 10 μ M) or ROCK inhibitor Y27632 (5 and 10 μ M) and followed for changes in cell shape and actin and vinculin immunostaining by phase contrast and confocal microscopy. In separate experiments, HTM cells were treated with TGF β 2 or CTGF for 24h in the presence or absence of 500nM AR-13324 and then assessed for induction of the fibrogenic markers α -smooth muscle actin (α -SMA), collagen1A and fibroblast specific protein-1 (FSP1) using immunofluorescence and immunoblotting analyses.

Results: AR-13324 induced significant cell shape changes in a time- and dose-dependent manner that correlated with a complete loss of actin stress fibers and focal adhesions. Compared to Y27632, AR-13324 was more potent in its effects on HTM cells. Treatment of HTM cells with 500nM AR-13324 for 24h decreased the basal levels of α -SMA, FSP1 and collagen1A and abolished the induction of these markers by TGF β 2 and CTGF.

Conclusions: AR-13324 induced cell shape changes, loss of actin stress fibers and focal adhesions in HTM cells. Additionally, AR-13324 significantly suppressed TGF β 2- and CTGF-induced levels of α -SMA collagen1A and FSP1, indicating an anti-fibrotic effect in HTM cells.

Effects of insulin on the porcine retinal arterioles and capillaries in a new model for studying the vascular diameter regulation in vitro

Peter S. Jensen¹, Toke Bek¹

¹*Department of Ophthalmology, Aarhus University Hospital*

Purpose: Disturbances in retinal blood flow secondary to impaired diameter regulation of retinal vessels are involved in the most frequent causes of blindness in the Western World. Therefore, the purpose of the present study was to develop an in vitro technique for studying diameter regulation in both larger and smaller retinal vessels and study the effects of insulin.

Methods: Porcine hemiretinas were mounted in a special tissue chamber developed for studying diameter regulation of vessels with different calibers while controlling temperature, pH and oxygen saturation. The chamber was positioned in a fluorescence microscope, and changes in the diameter of larger arterioles (25 μm or larger), pre-capillary arterioles (10-25 μm) and capillaries (smaller than 10 μm) were studied after intravascular and extravascular addition of insulin in both non-precontracted and precontracted vessels (n=6 for each variable) on altogether 12 hemiretinal segments.

Results: Insulin had no overall significant effect (intravascular $p>0.86$; extravascular $p>0.70$) on the diameter of non-precontracted vessels, whereas it dilated precontracted vessel of all three types, however only significantly for arterioles (intravascular $p<0.01$; extravascular $p<0.01$) and precapillary arterioles (intravascular $p<0.01$; extravascular $p<0.01$), but not capillaries (intravascular $p>0.07$; extravascular $p>0.31$). There was no significant difference between the three vessel types after intravascular addition of insulin ($p>0.06$), but after extravascular addition the dilating effect was significantly ($p<0.01$) more pronounced for pre-capillary arterioles than the two other vessel types.

Conclusions: Insulin appears to have no effect on non-precontracted vessels, but dilates precontracted vessels. The effect appears to differ between larger and smaller retinal vessels.

Endothelin B Receptor Mediated Neurodegeneration in a Rodent Model of Ocular Hypertension.

Raghu R. Krishnamoorthy, Alena Z. Minton and Shaoqing He

University of North Texas Health Science Center, Department of Cell Biology and Immunology, North Texas Eye Research Institute, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107

Purpose: ET_B receptors have been shown to contribute to neurodegeneration in animal models of glaucoma. Previous studies from our lab demonstrated increased RGC survival in ET_B receptor-deficient rats, compared to wild type rats following intraocular pressure (IOP) elevation. The purpose of this study was to understand mechanisms contributing to ET_B receptor mediated neurodegeneration *in vivo* in the Morrison's elevated IOP model of glaucoma in rats.

Methods: IOP elevation was carried out in one eye of adult wild type rats and ET_B receptor-deficient rats using the Morrison's method (by injection of hypertonic saline through episcleral veins), while the corresponding contralateral eye served as control. Following IOP elevation, rats were maintained for 2 weeks and sacrificed. Retinal sections were obtained from control and IOP elevated rat eyes and analyzed for changes in immunostaining for c-Jun and Bax by immunohistochemistry. In separate experiments, purified rat retinal ganglion cells (RGCs) in culture were treated with ET-1 for 24 hr and immunocytochemical analysis of c-Jun, phospho-c-Jun and Bax was carried out using specific antibodies.

Results: IOP elevation produced an increase in immunostaining for both c-Jun and Bax in the retinal ganglion cell layer in wild type rats, which was attenuated in the ET_B receptor-deficient rats. Purified RGCs in culture showed increased immunostaining for c-Jun, phospho-c-Jun and Bax following ET-1 treatment for 24 hr.

Conclusions: The immediate early gene c-Jun, a component of the AP-1 family of transcription factor may be involved in mediating ET_B receptor's effect on neurodegeneration through apoptotic pathways including Bax.

Role of HIF-1 α in retinal ganglion cell death

Shahid Husain, Sudha Singh, and Anis Ahmad

Department of Ophthalmology, Medical University of South Carolina, Charleston, SC 29425

Purpose: To determine the role of HIF-1 α in RGC death during glaucomatous injury

Methods: Brown Norway rats were used to elevate intraocular pressure by injecting 50 μ L of 2M hypertonic saline into limbal veins. Pattern electroretinograms (PERG) and retinal ganglion cells (RGCs) in flat mount were counted 4-6 week post injury. The changes in the expression patterns of HIF-1 α were determined by immunohistochemistry. To assess hypoxic regions in the glaucomatous retina, we have used the commercially available HypoxyprobeTM-1 kit, which contains pimonidazole HCl and anti-pimonidazole antibodies.

Results: Although the mechanisms of RGC death in response to glaucomatous injury are not clearly defined, some evidence suggests that tissue hypoxia may adversely affect RGC survival via pro-apoptotic pathways. Our data show an up-regulation of HIF-1 α in the inner retinal layers on day 7-28, post glaucomatous injury. The pimonidazole staining was clearly increased in NFL, INL and RGCs, and confirming the presence of hypoxic components, when compared with the contralateral normal eye. To determine functional changes associated with glaucomatous injury, we measured RGC function by pattern-electroretinogram (pattern-ERG). The pattern-ERGs were not changed at day 7 post injury, while HIF-1 α and pimonidazole staining were increased. In contrast, pattern-ERG amplitudes were reduced by 25% by day 28 post injury, while HIF-1 α still remained significantly up-regulated.

Conclusion: These data form a strong rationale that hypoxia develops very early during glaucomatous injury, which subsequently up-regulates HIF-1 α and other neurotoxic proteins which may have promoted RGC death during glaucomatous injury.

AMPA Receptor Dual Role in RGC Survival

Yong H. Park^{1,2}, Nolan R. McGrady^{2,3}, Thomas Yorio^{1,2}

¹Pharmacology and Neuroscience, University of North Texas Health Science Center, Fort Worth, TX, USA

²North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, TX, USA

³Cell Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX, USA

Purpose: The purpose of this study was to investigate if AMPAR desensitization attenuates excitotoxicity in purified retinal ganglion cells (RGCs) under normoxic and hypoxic conditions.

Methods: Purified RGCs were cultured for 7 days in vitro before series of AMPAR agonists (100 μ M s-AMPA (desensitizing), 100 μ M kainic acid (non-desensitizing)), and an AMPAR modulator (100 μ M cyclothiazide) for 72h in RGC defined medium. To determine if excitotoxicity occurs following hypoxic injury, RGCs were incubated in 0.5% O₂ with DMEM/without glucose (OGD) for 4h, following which the cells were treated with s-AMPA in OGD for an additional 4h. A Live-Dead Assay determined cell viability.

Results: Significantly enhanced viability was found in RGCs treated with 100 μ M s-AMPA (84 $\pm$ 1% viable) compared to vehicle (0.1% DMSO) group (71 $\pm$ 4% viable) alone (p<0.05). Treatments with s-AMPA in combination with cyclothiazide or kainic acid significantly reduced cell viability to 50 $\pm$ 3% and 54 $\pm$ 2%, respectively (p<0.01). A marked decrease in RGC survival was observed with cells treated with s-AMPA following OGD injury compared to normoxic glucose deprivation condition. However, no significant decrease in RGC survival was observed when OGD was carried out for 6h in the presence of s-AMPA in the cell culture medium.

Conclusions: Desensitization of AMPAR is a key determinant of s-AMPA-mediated excitotoxicity, whereby blocking the desensitization of AMPAR induces cell death. The desensitizing agonist, s-AMPA, increases susceptibility to excitotoxicity following OGD in RGCs. Future studies will determine AMPAR subunits with greater ion current influx mediate increased sensitivity to excitotoxicity following injury.

Differential control of intracellular calcium signaling in primary adult rat optic nerve head astrocytes.

Yuliya Naumchuk,¹ Andrew J. Payne,¹ Krupa R. Patel,¹ Simon Kaja,¹ Peter Koulen^{1,2}

¹Department of Ophthalmology, Vision Research Center, and ² Department of Basic Medical Science, University of Missouri – Kansas City, School of Medicine, 2411 Holmes St., Kansas City, MO 64108, USA

Purpose: Pathological changes to optic nerve head astrocytes (ONHAs) are a hallmark of glaucomatous retinopathy, and include activation, migration, extracellular matrix remodeling and altered gene and protein expression. Yet only little is known regarding the intracellular signaling pathways in ONHAs. We herein conducted a detailed quantitative analysis of intracellular Ca^{2+} signaling in ONHAs.

Methods: We optimized the culture conditions for adult rat ONHAs and performed a detailed immunocytochemical analysis of the expression and subcellular distribution of intracellular Ca^{2+} channels. Optical imaging of the intracellular Ca^{2+} concentration was used to determine the channel-specific contributions to stimulus-induced Ca^{2+} release from intracellular stores.

Results: We identified strong immunoreactivities for type 1 and type 2 inositol,1-4-5,-trisphosphate receptors (IP_3Rs) in the endoplasmic reticulum (ER) and the nucleus, respectively. We did not detect any type 3 IP_3R immunoreactivity in primary cultured ONHAs. All ryanodine receptor (RyR) subtypes showed strong immunoreactivity. We observed significant responses to pharmacological stimulus-induced intracellular Ca^{2+} release from both IP_3Rs and RyRs . Subcellular quantification revealed differential nuclear vs. cytosolic IP_3R -mediated Ca^{2+} release, in accordance with type 2 IP_3Rs as the major contributor to intracellular Ca^{2+} release.

Conclusions: ONHAs utilize differentially distributed intracellular Ca^{2+} channels in order to maintain Ca^{2+} homeostasis. Our data provide the critical foundation for future studies investigating Ca^{2+} signaling changes in ONHAs in glaucomatous retinopathy. Furthermore, our optimized protocol for primary culture of adult rat ONHAs provides new feasibility data for using ONHAs for drug discovery for glaucomatous retinopathy and related disorders affecting the optic nerve and optic nerve head.

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Human Aqueous Humor Exosomes

Yutao Liu¹, W. Michael Dismuke², Pratap Challa², Iris Navarro², W. Daniel Stamer^{2,3},

¹*Department of Cellular Biology and Anatomy, Medical College of Georgia, Georgia Regents University, Augusta, GA*

²*Department of Ophthalmology; 3 Department of Biomedical Engineering, Duke University Medical Center, Durham, NC*

Conflict of Interest: None for all the authors

Keywords: aqueous humor, glaucoma, exosome, exosomal RNA, miRNA

Aqueous humor (AH) is a dynamic intraocular fluid that supports the vitality of tissues regulating intraocular pressure. We recently discovered that extracellular nanovesicles called exosomes are a major constituent of AH. Exosomes function in extracellular communication and contain proteins and small RNA. Our goal was to characterize the physical properties of AH exosomes and their RNA (esRNA) content. We isolated exosomes from human AH collected during cataract surgery from five patients using serial ultracentrifugation. We measured the size and concentration of AH exosomes in solution using nanoparticle tracking analysis. We found a single population of vesicles having a mean size of 121 ± 11 nm in the unprocessed AH. Data show that centrifugation does not significantly affect the mean particle size (121 ± 11 nm versus 124 ± 21 nm), but does impact the final number of exosomes in solution (87% loss from the unprocessed AH; n=5). We extracted esRNA from the pooled human AH samples using miRCURY RNA isolation kit from Exiqon. The quality of extracted esRNA was evaluated using Agilent Bioanalyzer 2100 and was used to generate a sequencing library for small RNA sequencing. More than 10 different miRNAs were identified; abundant species included miR-486-5p, miR-204, and miR-184. We found that the majority of extracellular vesicles in the AH were in the exosome size range, suggesting that miRNAs housed within exosomes may function in communication between AH inflow and outflow tissues. In summary, for the first time we demonstrate that exosomes are the major extracellular vesicle population in AH and that these exosomes contain characteristic esRNAs.

Improvement of outcome measures of dry eye by a novel integrin antagonist in the murine desiccating stress model

Achim H. Krauss¹; Rosa M. Corrales²; Flavia S. A. Pelegrino²; Johanna Tukler-Henriksson²; Edit Kurali³; Stephen C. Pflugfelder²; Cintia S. de Paiva²

¹GSK Ophthalmology, ³Statistical Consulting Group, Quantitative Sciences, King of Prussia, PA, USA; ²Ocular Surface Center, Dept. of Ophthalmology, Baylor College of Medicine, Houston, TX, USA

Purpose: GW559090 is a novel, competitive and high-affinity $\alpha 4\beta 1$ integrin antagonist. Through interaction with VCAM-1 and fibronectin $\alpha 4\beta 1$ integrin is involved in leukocyte trafficking and activation. This study investigated the effects of GW559090 in a murine model of dry eye.

Methods: All studies were conducted according to the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals after review by the GSK and BCM Institutional Animal Care and Use Committees. Female C57BL/6 mice, aged 6 to 8 weeks, were subjected to desiccating stress (DS). Bilateral topical BID treatment with GW559090 was compared to vehicle treated controls. Treatment was initiated at the time of DS induction. Treatment effects were assessed on corneal staining with Oregon Green Dextran (OGD); conjunctival T cell infiltration by immunohistochemistry; expression of inflammatory markers in ocular surface tissues by real time PCR.

Results: Topical GW559090 significantly reduced corneal uptake of OGD compared to vehicle-treated disease controls in a dose dependent manner (1mg/ml; 3mg/ml; 10mg/ml; 30mg/ml) with 30mg/ml showing the greatest reduction in OGD staining ($p < 0.01$). This effect was similar to inhibition of OGD staining achieved with topical dexamethasone. A significant decrease in the number of infiltrating CD4⁺ T cells in the conjunctival epithelium was observed in the GW559090-treated group compared to disease control ($p < 0.001$). Conjunctival expression of IL-1 α ($p < 0.001$) and CCL20 ($p < 0.05$) was reduced in GW559090 treated eyes.

Conclusion: GW559090, a novel $\alpha 4\beta 1$ integrin antagonist, improved outcome measures of corneal staining, infiltrating CD4⁺ T cells and conjunctival IL-1 α and CCL20 expression in this murine model of dry eye. These results indicate the potential of this novel agent for the treatment of dry eye disease.

A Novel approach for expansion of epithelial stem cells on contact lenses: Towards cell therapy treatment of ocular surface diseases.

Ariel Gore¹, Vered Horwitz¹, Orit Cohen-Jacob¹, Hila Gutman¹, Mayan Cohen¹, Liat Cohen¹, Josef Turetz¹, Shlomit Dachir¹ and Tamar Kadar¹.

¹*Department of Pharmacology, Israel Institute for Biological Research, Ness Ziona, Israel*

Purpose: The aim of the present study was to evaluate the potential use of contact lenses (CLs) as a carrier of limbal stem cells (LSC) to the ocular surface, replacing current methods involving biological materials.

Methods: Limbal epithelial cells were isolated from rabbit cornea and cultured with 3T3 cells on CLs. The preservation of LSC phenotype was determined using p63 α and ABCG2 immunostaining, whereas epithelial differentiation was evaluated using CK3 and CK19. CLs seeded with PKH26-labeled LSCs were transferred to rabbit eyes after performing a surgical keratectomy, and the transition and phenotype were evaluated in whole-mount corneas while re-epithelialization was determined by slit-lamp microscope and fluorescein follow up.

Results: Proliferation of individual limbal cells was observed on CLs with a 3T3 feeder cell layer, showing holoclone formation and retention of viable stem cell phenotype. A higher transition of cultivated cells after a dual sequential CL transplantation to the ocular surface was observed, showing the preservation of the LSC phenotype in the corneal surface. Finally, an enhanced re-epithelialization was observed in the transplanted animals compared to the sham-transplanted.

Conclusions: CLs containing LSC were shown as an ideal carrier for transferring cells to the ocular surface due to SC phenotype preservation, cell transition and friendly user replacement. This novel technique of using CLs as a carrier offers an easily manipulable and nonimmunogenic method for transferring LSCs for ocular surface reconstruction in patients, thus, replacing existing biological methods and reducing the possibility of infections.

Effects of amitriptyline on the spontaneous and stimulus-evoked activity of corneal cold-sensitive nerve terminals.

Juana Gallar¹, Takayoshi Masuoka², Carlos Belmonte¹

¹*Instituto de Neurociencias, Universidad Miguel Hernandez-CSIC, San Juan de Alicante, Alicante, Spain.*

²*Department of Pharmacology, Kanazawa Medical University, Uchinada, Ishikawa, Japan.*

Purpose: To determine the effect of Na⁺-channel blocker amitriptyline on the spontaneous and stimulus-evoked electrical activity of corneal cold nerve terminals recorded from corneas of control and tear-deficient guinea-pigs.

Methods: Corneal cold-sensitive nerve terminal impulse (NTI) activity was recorded from the isolated cornea mounted in a recording chamber superfused at 34°C, 4-6 weeks after removal of the exorbital lacrimal gland in the anesthetized guinea-pig. Thermal stimulation was performed changing the perfusion solution temperature from 34°C to 20°C (cooling ramp) or to 50°C. The characteristics of the spontaneous and stimulus-evoked NTI activity recorded from dry eye (DE) and intact corneas before and during perfusion with 10-30µM amitriptyline were compared.

Results: Amitriptyline reduced irreversibly the spontaneous NTI activity at 34°C in a dose-dependent manner in both intact and DE corneas. Inhibition of spontaneous NTI by 10µM amitriptyline was significantly more effective in intact corneas than in DE, where 42% of units were insensitive to amitriptyline; these amitriptyline-insensitive units presented higher cooling thresholds than amitriptyline-sensitive units. Amitriptyline tends also to decrease the maximal response to cooling ramps in intact corneas but not in DE corneas.

Conclusions: Na⁺-channel blocker amitriptyline reduces the spontaneous activity and cooling response of cold nerve terminals in intact corneas and in a lesser degree in DE corneas. This difference may be due to the changes induced by chronic ocular dryness in the expression of the various voltage-sensitive Na⁺ channels responsible for impulse generation in corneal cold-sensitive terminals.

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Mesenchymal Stem Cells (MSCs) inhibit Bacterial induced corneal inflammation

Rui Zhang¹, Yan Sun², Eric Pearlman², Suber S Huang¹

¹University Hospitals Eye Institute, Case Western Reserve University, Cleveland, OH

²Department of Ophthalmology and Visual Sciences, Case Western Reserve University, Cleveland, OH

Purpose: This study evaluates whether the targeted delivery of mesenchymal stem cells (MSCs) decreases the amount of pathologic and inflammatory response to ocular injury using a bacterial infection model.

Methods. Infection of C57BL/6 mice corneas was induced by *Pseudomonas aeruginosa*. The mice were then divided into 3 groups. One group received topical moxifloxacin 2 hrs post procedure, then every 12 hrs for 24 hrs; one group received Human-derived MSCs injected into the subconjunctival space, and the third group received topical moxifloxacin and subconjunctival MSCs. The mice were sacrificed on day 7, and the degree of corneal infiltration and inflammation was studied via confoscan and histological sections.

Results: The corneas of mice treated with MSCs and antibiotic displayed less stromal edema, infiltration and clinical disease on day 2 and 5 after treatment. Confoscan data at day 7 showed that mice treated with MSCs and antibiotic had significantly less stromal haze and thickness with a $P < 0.05$ when compared to mice treated with antibiotics. Clinical photos taken on day 7 also displayed less disease, scarring, and inflammation in mice treated with MSCs and antibiotic versus antibiotic alone.

Conclusion: These results suggest that the local delivery of MSCs may be an effective and safe way to supplement and immunomodulate the ocular inflammatory process driven by the innate and adaptive immune responses. MSCs have little immunogenicity, do not require HLA typing, and have already been used intravenously in humans without serious consequences. These results hold promise for future clinical trials using MSCs in ocular keratitis and other inflammatory diseases.

Mechanisms involved in azithromycin's stimulation of human meibomian gland epithelial cell function.

Yang Liu, Juan Ding, Wendy R. Kam and David A. Sullivan.

Schepens Eye Research Institute, Massachusetts Eye and Ear, Harvard Medical School, Boston, MA, USA

Purpose: We recently discovered that azithromycin (AZM) acts directly on human meibomian gland epithelial cells (HMGEs) to stimulate their differentiation and enhance their lipogenesis. We also discovered that AZM increases the levels of free cholesterol, cholesterol ester and phospholipids, and the formation of lipid-filled lamellar lysosomes. We hypothesize that AZM's phospholipidosis-like effect is due to its cationic amphiphilic drug (CAD) nature. In addition, we hypothesize that AZM's influence is mediated, at least in part, through an upregulation of sterol regulatory element-binding protein (SREBP-1), a transcription factor that plays a very important role in the regulation of cholesterologenic and lipogenic enzymes. To test these hypotheses, we examined whether AZM stimulates SREBP-1 protein expression in HMGEs, and whether the non-CAD antibiotics (i.e. doxycycline, minocycline or tetracycline) can duplicate any AZM effect.

Methods: Immortalized HMGEs were cultured in the presence of vehicle, AZM, doxycycline, minocycline or tetracycline (10 µg/ml) for 5 days. SREBP-1 content in cellular lysates was analyzed by Western blot. Cells were also evaluated for the appearance of lysosomes (LysoTracker), neutral lipid (LipidTox) staining, and lipid composition (high performance thin-layer chromatography).

Results: Our findings demonstrate that AZM significantly increases SREBP-1 levels in HMGEs and that this action is not reproduced by the non-CAD antibiotics. Further, doxycycline, minocycline or tetracycline treatment did not duplicate AZM's ability to stimulate the accumulation of cholesterol, cholesterol esters, phospholipids and lysosomes in HMGEs.

Conclusions: Our results support our hypotheses and indicate that AZM's CAD-like effects on HMGEs are unique, as compared to those of other antibiotics.
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Effective diagnosis in inclusion conjunctivitis in Colombia and Azithromycin as topical treatment

Sandra Johanna Garzon Parra

Introduction: Ocular infection with Chlamydia Trachomatis causes follicular keratoconjunctivitis and trachoma, the first cause of blindness in the world. The World Health Organization designed the Global Alliance to Eliminate Blinding Trachoma by 2020 plan, Colombia no was included because apparently was not in risk. But also, from 2010 a group of physicians in Vaupes, a forest region, reported in three poor communities active trachoma. The beneficial diagnosis was successful by detailed exam and treatment.

Purpose: Describe the importance of early detection and intervention of ocular signs in trachoma.

Methods: Literature review in Colombian reported cases of trachoma. Searched databases: Medline, Cochrane, lilacs. Also contacted investigators and experts in the field.

Results: Clinical signs as follicles on the tarsal conjunctiva, inflammatory thickening obscuring more than half of the normal deep tarsal vessels, Arlt's line, easily visible white bands of scars and trichiasis was recognized to identify ocular trachoma. Intervention included face washing combined with topical Azithromycin to reducing active trachoma.

Conclusions: There is some evidence that early detection and intervention can reduce the disease evolution. Azithromycin generates inhibition of protein synthesis in cell replication of chlamydia trachomatis, stopping the ocular active infection.

“The effect of Topical Dexamethasone γ -Cyclodextrin Nanoparticle Eye Drops in diabetic macular edema”

Akihiro Ohira¹ M.D., Ph.D., Katsunori Hara¹ M.D., Gauti Jóhannesson² M.D., Ph.D.; Masaki Tanito¹ M.D., Ph.D.; Gudrún Marta Ásgrímsdóttir³ M.Pharm.; Sigrún H. Lund⁴ Ph.D.; Thorsteinn Loftsson⁵ Ph.D.; Einar Stefánsson⁶ M.D., Ph.D

¹*Department of Ophthalmology, Shimane University Faculty of Medicine, Izumo, Shimane, Japan;*

²*Department of Clinical Science, Ophthalmology, Umeå University, Umeå, Sweden*

³*Oculus ehf. Reykjavik, Iceland*

⁴*Centre of Public Health Sciences, Faculty of Medicine, University of Iceland, Reykjavik, Iceland*

⁵*Faculty of Pharmaceutical Sciences, University of Iceland, Reykjavik, Iceland*

⁶*Department of Ophthalmology, Faculty of Medicine, National University Hospital, University of Iceland, Reykjavik, Iceland*

Purpose: To test in a randomized prospective study the efficacy and safety of topical 1.5% dexamethasone γ -cyclodextrin nanoparticle eye drops (dexNP) for diabetic macular edema (DME) and compare to posterior subtenon injection of triamcinolone acetonide.

Material and methods: In a prospective randomized controlled trial 22 eyes of 22 consecutive patients with chronic DME were randomized to a) topical treatment with dexNP x3/day for one month, x2/day the next month and finally x1/day the third month or b) one posterior subtenon injection of 20mg triamcinolone acetonide. Study visits included best-corrected visual acuity, intraocular pressure (IOP), spectral domain optical coherent tomography and blood samples at baseline and at 4,8,12 and 16 weeks.

Results: The logMAR visual acuity improved significantly with dexNP from 0.41 ± 0.3 (mean $\pm$ SD) to 0.32 ± 0.25 and 0.30 ± 0.26 at 4 and 8 weeks respectively. One third of the eye drop group improved more than 0.3 logMAR units. For triamcinolone, logMAR changed significantly from 0.42 ± 0.28 at baseline to 0.32 ± 0.29 at 4 weeks and 0.33 ± 0.37 at 12 weeks. The central macular thickness (CMT) decreased significantly with dexNP from 483 ± 141 mm to 384 ± 142 mm at 4 weeks and 342 ± 114 mm at 8 weeks. For triamcinolone, CMT decreased significantly at all time points. : 494 ± 94 mm, 388 ± 120 , 388 ± 145 , 390 ± 136 and 411 ± 104 mm at 0, 4, 8, 12 and 16 weeks respectively. There was a modest increase in IOP at all time points with dexamethasone nanoparticle eye drops while no increase was seen with triamcinolone. Serum cortisol was affected by both treatments

Conclusion: Topical dexNP significantly improve visual acuity and decrease macular thickness in patients with DME. The effect is similar to that from subtenon triamcinolone as well as to reports on intravitreal steroid implants (Ozurdex[®]) and triamcinolone intravitreal injections. A modest increase in IOP was seen with the nanoparticle eye drops but IOP normalized after discontinuation of treatment.

A Clickable Dendrimer Hydrogel Platform for Ocular Drug Delivery

Hu Yang^{1,2}, Leyuan Xu¹, Olga Yu. Zolotarskaya¹, Sudha Singh³, Shahid Husain³, Christopher T. Leffler, Uday B. Kompella⁵

¹*Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, Virginia, United States*

²*Massey Cancer Center, Virginia Commonwealth University, Richmond, Virginia, United States*

³*Department of Ophthalmology, Medical University of South Carolina, Charleston, South Carolina, United States*

⁴*Department of Ophthalmology, Virginia Commonwealth University, Richmond, Virginia, United States*

⁵*Department of Pharmaceutical Sciences, University of Colorado, Anschutz Medical Campus, Aurora, Colorado, United States*

Purpose: The purpose of this work is to develop a clickable dendrimer hydrogel (cDH) platform using copper-free click chemistry and apply this platform topically to deliver antiglaucoma drugs.

Methods: The platform preparation involves synthesis of dendrimer conjugates carrying complementarily reactive dibenzocyclooctyne and azide functional groups, respectively, and in situ copper-free click reaction in the presence of antiglaucoma drugs. Cytotoxicity of cDH to primary human corneal epithelial (HCET) cells and primary human retinal pigment epithelial (HRPE) cells was assessed by using the WST-1 assay. Extracellular release of pro-inflammatory mediator interleukin-1 α (IL-1 α) levels into HCET medium was quantified with ELISA. Retinal ganglion cells (RGC) structure and functions in Brown Norway rat eyes at 7 days following topical administration were measured. Intraocular pressure (IOP) was monitored throughout the experiment.

Results: cDH was found to be cytocompatible with HCET at 2.5 μ M or below and cytocompatible with HRPE up to 45 μ M. The preliminary test did not show increased release of IL-1 α , indicating negligible irritation of cDH. Over 7 days, no eye irritation was observed. IOP, RGC integrity, and pattern electroretinogram remained unchanged when compared to baselines (day 0 prior to cDH administration). Spectral domain ocular coherence tomography (OCT) also indicated that retinal structure was not affected by treatment.

Conclusions: Our preliminary work supports the topical use of cDH. This new platform has potential to develop efficient personalized topical drug formulations for glaucoma treatment.

Dexamethasone-Eluting Contact Lens

Joseph B. Ciolino^{1,2,3}, Borja Salvador^{1,2,3}, Rekha Tulsan^{1,2,3}, Amy E. Ross^{1,2,3},
Daniel S. Kohane^{2,3}

¹Massachusetts Eye and Ear Infirmary, Boston, MA 02114, USA

²David H. Koch Institute for Integrative Cancer Research, 77 Massachusetts Ave, Building 76-66, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

³Laboratory for Biomaterials and Drug Delivery, Department of Anesthesiology, Division of Critical Care Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115, USA.

Purpose: To report the *in vivo* testing of a dexamethasone-eluting contact lens designed for the treatment of ocular inflammation.

Methods: Drug-eluting therapeutic contact lenses (TCL) were created by encapsulating dexamethasone-Poly(lactic-co-glycolic acid) films in methafilcon A by ultraviolet light polymerization. TCLs were inserted on the left eye of New Zealand white rabbits (n=4) for 7 days. The eyes were examined under an operating microscope and the anterior chamber (AC) fluid was collected each day over the course of one week of continuous wear. TCL's were placed on the left eye of rabbits, which were euthanized 6 hours or 24 hours later. Drug concentrations were measured by high-performance liquid chromatography in combination with high-resolution mass spectrometry (LC-MS).

Results: *In vivo*, the TCLs demonstrated no signs of toxicity. The TCL demonstrated a large early burst with followed by a steady state delivery rate that remained well above therapeutic levels for one week. Through 4 days of continuous wear, TCLs delivered more drug to the anterior chamber each day than dexamethasone drops administered every hour. After 24 hours of TCL, tissue drug concentrations (ng/g) were equal to or greater than reported values for hourly drops for all tissues tested: cornea ($9,381 \pm 154$), sclera (734 ± 170), aqueous humor ($1,224 \pm 190$), iris ($2,268 \pm 306$), vitreous (91 ± 25), retina (934 ± 638), and choroid (667 ± 149).
Conclusions: This contact lens design can potentially be used as a treatment for ocular inflammation and as a platform for ocular drug delivery with widespread applications.

The effect of hydroxypropyl-guar gellable lubricant eye drops on tear film stability and corneal sensitivity in patients with dry eye

Lóránt Dienes¹, Huba J Kiss¹, Zsuzsanna Szepessy¹, Zoltán Z Nagy¹, M.Carmen Acosta², Juana Gallar², Illés Kovács¹

¹Semmelweis University, Department of Ophthalmology, Budapest, Hungary

²Instituto de Neurociencias, Universidad Miguel Hernandez-CSIC, San Juan de Alicante, Spain.

Purpose: To investigate the effect of improved tear film stability on corneal sensitivity to selective stimuli in dry eye patients.

Method: This study included twenty patients with dry eye symptoms. Tear film dynamics was assessed by non-invasive tear film breakup time (NI-BUT) in parallel with continuous recordings of irritation scores during forced blinking using a rotating potentiometer. Irritation responses to selective stimulation of corneal mechano, cold and polymodal receptors were assessed using the Belmonte gas esthesiometer. All the measurements were made before and 5 min after application of one drop of a hydroxypropyl-guar gellable lubricant eye drop.

Results: NI-BUT significantly increased after tear supplementation (before: 8.18 ± 3.28 vs. after: 10.44 ± 4.44 sec; $p < 0.001$). The intensity of irritation increased significantly during forced blinking (quadratic regression $r^2 = 0.89$, $p < 0.001$) both before and after artificial drops. Tear supplementation significantly decreased irritation scores ($p = 0.004$) and lowered the proportion of patients experienced maximum irritation through the interblink interval ($p < 0.05$). After tear supplementation, irritation scores to all selective stimulations decreased significantly ($p < 0.05$).

Conclusion: Tear supplementation significantly improved tear film stability and decreased ocular irritative responses, however the sudden increase of irritation after blinking suggests altered excitability of corneal receptors in dry eye. These results demonstrate that improved tear film dynamics helps to alleviate dry eye symptoms through a direct beneficial effect on the protective layer of the precorneal tear film but does not have an effect on the excitability of corneal nerves.

Small Chain Triglycerides as Novel Platform for Ocular Drug Delivery of Protein Therapeutics

R. Michael Burr¹, Moritz Vollrath², Gerhard Winter², Bala K. Ambati¹

¹*Moran Eye Center, University of Utah, SLC, Utah, USA*

²*Dept. of Pharmaceutical Technology, Ludwig Maximilian University, Munich, Germany*

Purpose: Treatment of wet macular degeneration is limited in its delivery method with a high frequency of intraocular injections posing significant risks to patients. A novel drug delivery system that limits the number of ocular injections would be of great benefit to patients and clinicians. Herein a novel method for controlled release of anti-VEGF therapies is proposed that uses small chain triglycerides (SCTs) manufactured with hot-melt extrusion. This method eliminates the need for organic solvents, is done at protein friendly temperatures below 40 °C, and provides zero-order kinetic release of proteinaceous drugs up to 6 months.

Method: Annular shaped lipid extrudates were implanted in the ciliary sulcus of female New Zealand White Rabbits and were observed by clinical examination for 3 months with H&E done postmortem. Human cataract extracts were compared with blank rabbit vitreous, aqueous humor, and lens tissue to quantify lipase activity using the Lipase Activity Colormetric Assay Kit (Biovision).

Results: SCTs between chain length 12 and 18 degrade in rabbit eyes with no evidence of inflammation. *Ex vivo* human cataract extracts possess lipase activity and degrade SCTs. Afibrilcept, bevacizumab, and ranibizumab were screened as candidate drugs for this platform technology.

Conclusion: SCTs have a good ocular biocompatibility when implanted in the ciliary sulcus. Lipases present in the anterior chamber could be the driving force in lipid degradation. Ranibizumab is a good candidate to use in a lipidic drug delivery system. These data warrant further exploration into the use of lipids as a controlled release agent of proteins for ocular applications.

The bioavailability of slow release flurbiprofen after intravitreal administration in the rabbit eye.

Tsilimbaris MK¹, Tsika C¹, Tsiapa I¹, Pachis K², Blazaki S², Tzatzarakis M³, Antimisiaris SG²

¹*Department of Ophthalmology, University of Crete, Heraklion, Greece*

²*Laboratory of Pharmaceutical Technology, Department of Pharmacy, University of Patras, Rio, Greece*

³*Center of Toxicology Science & Research, Medical School, University of Crete, Heraklion, Greece*

Purpose: To evaluate the intravitreal bioavailability of a sustained release system of the Non Steroidal Anti-inflammatory Drug Flurbiprofen sodium.

Methods: We created a sustained release system of flurbiprofen sodium with concentration: 7.66mg/ml. We injected intravitreally 0.1ml of this solution in the right eye (OD, study eye) of the experimental rabbits. The left eye (OS) received 0.1ml of flurbiprofen sodium in PBS (C=7.66mg/ml). We compared the kinetics of the slow release flurbiprofen with the flurbiprofen alone. We measured the intravitreal concentration at time points 0, 24 hours, 72hours and 168hours (7days) in 3 animals per time point with a HPLC/MS method.

Results: No adverse events were observed during the study. No signs of inflammation, hemorrhage or detachment were detected. The recovery of the method was 92.6%. The clearance of flurbiprofen follows first order kinetics. The half-life of the slow release flurbiprofen solution was 24.75 hours while the half-life of the flurbiprofen alone was 16.12 hours. The elimination constant rate (K) was 0.028 and 0.043 respectively.

Conclusions: We managed to extend the presence of the drug into the vitreous cavity. This is crucial for drugs such as the NSAIDs that have a very short intravitreal half-life time. However, the need for greater prolongation in the vitreous demands further experimentation with the system's structure and the substance's dosage.

AAV-mediated antisense oligonucleotide delivery is an effective therapeutic approach for CEP290-associated LCA

Rob W.J. Collin^{1,2}, Lonneke Duijkers¹, Ru Xiao³, Jean Bennett⁴, Luk H. Vandenberghe³ and Alejandro Garanto^{1,2}

¹Department of Human Genetics and ²Radboud Institute for Molecular Life Sciences, Radboud University Medical Centre, Nijmegen, The Netherlands

³Ocular Genomics Institute, Schepens Eye Research Institute, Massachusetts Eye and Ear Infirmary, Department of Ophthalmology, Harvard Medical School, Harvard University, Boston

⁴F.M. Kirby Center for Molecular Ophthalmology and Center for Advanced Retinal and Ophthalmic Therapeutics, Scheie Eye Institute, University of Pennsylvania School of Medicine, Philadelphia, PA, USA.

Leber congenital amaurosis (LCA) is a genetically heterogeneous disorder characterized by severe visual impairment starting in the first year of life. The most frequent genetic cause of LCA, present in up to 15% of all LCA cases in some Western populations, is an intronic mutation in *CEP290* (c.2991+1655A>G) that creates a cryptic splice donor site and results in the insertion of an aberrant exon into *CEP290* mRNA. Previously, we have shown that antisense oligonucleotides (AONs) effectively restore normal *CEP290* splicing in patient-derived lymphoblastoid cells. Given the safety and efficacy of adeno-associated viruses (AAVs) used in ongoing clinical trials for other genetic subtypes of retinal dystrophy, we here aimed to explore the therapeutic potential of AAV-based delivery of AONs. Transduction of patient-derived fibroblast cells with effective AONs cloned into a modified U7snRNA construct and packaged into AAV2/2 fully restored normal *CEP290* pre-mRNA splicing and significantly increased *CEP290* protein levels. Moreover, a ciliary phenotype present in these fibroblasts was completely rescued upon transduction of AON-containing AAVs. Together, our data show that AAVs are an excellent therapeutic vector for the delivery of AONs to restore splice defects, and highlight the tremendous potential of AONs for the treatment of *CEP290*-associated LCA.