Association for Ocular Pharmacology and Therapeutics

8th Scientific Meeting

February 9-11, 2007
San Diego, California

Association for Ocular Pharmacology and Therapeutics

UNIVERSITY *of* CALIFORNIA
SAN DIEGO
SCHOOL OF MEDICINE

Sponsored by

Association for Ocular Pharmacology and Therapeutics
University of California, San Diego Continuing Medical Education

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for their generous educational grants.

Their financial support has made this conference possible.

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Association for Ocular Pharmacology and Therapeutics
8Th Scientific Meeting

~Target Audience~

This program is designed for principal investigators, scientists, medical professionals, technicians, and ophthalmic-care providers.

~Objectives~

- *Discuss new developments in medical topics focusing on issues specific to ophthalmic pharmacology*
- *Review ophthalmic treatment paradigms to enhance management of patients with chronic ophthalmic conditions such as glaucoma, age-related macular degeneration, diabetes, myopia and ocular surface disease*
- *Expand the scope of care to encompass contemporary laboratory investigations with potential patient-care implications*

~Mission Statement~

The mission of the **Association for Ocular Pharmacology and Therapeutics (AOPT)** is to serve as a global forum for the exchange of information about ocular pharmacology to meet the needs of vision scientists and eye care professionals for the advancement of vision research and the treatment of ophthalmic disorders worldwide.

Our vision for this organization is to be recognized throughout the world as the premier scientific society and forum for pharmacology of the eye. AOPT uses innovative techniques for publication and dissemination of research findings through its journal, the Journal of Ocular Pharmacology and Therapeutics, and biennial meetings. AOPT has a diverse leadership and membership and is recognized for its collegiality, responsiveness, and community spirit.

The Mission of the **University of California, San Diego, Office of Continuing Medical Education (UCSD CME)** is to provide relevant postgraduate educational opportunities to physicians and other health care providers that maintain or produce changes in knowledge, skills, attitudes or behaviors to nurture and enable the optimum provision of health care.

We are committed to continual improvement of our educational mission by researching current adult education and CME literature, participation in national organizations committed to improving CME, and implementation of innovative and creative programming that may further our mission.

~Accreditation~

This activity has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education ("ACCME") through the joint sponsorship of the University of California, San Diego School of Medicine and the Association for Ocular Pharmacology and Therapeutics. The University of California, San Diego School of Medicine is accredited by the ACCME to provide continuing medical education for physicians.

The University of California, San Diego School of Medicine designates this educational activity for a maximum of 22.0 **CREDITS** *AMA PRA Category 1 Credit(s)*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

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Association for Ocular Pharmacology and Therapeutics 8th Scientific Meeting
February 9-11, 2007

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The following speakers have no relevant financial relationships to disclose:

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POSTERS
FRIDAY, FEBRUARY 9, 2007

POSTERS
SATURDAY, FEBRUARY 10, 2007

UNIVERSITY of CALIFORNIA, SAN DIEGO
SCHOOL OF MEDICINE

CALIFORNIA ASSEMBLY BILL 1195
Cultural and Linguistic Competency

Supplement for UCSD CME Programs

California Assembly Bill 1195 requires continuing medical education activities with patient care components to include curriculum in the subjects of cultural and linguistic competency. It is the intent of the bill, which went into effect on July 1, 2006, to encourage physicians and surgeons, CME providers in the state of California, and the Accreditation Council for Continuing Medical Education to meet the cultural and linguistic concerns of a diverse patient population through appropriate professional development.

Cultural competency is defined as a set of integrated attitudes, knowledge, and skills that enables health care professionals or organizations to care effectively for patients from diverse cultures, groups, and communities. Linguistic competency is defined as the ability of a physician or surgeon to provide patients who do not speak English or who have limited ability to speak English, direct communication in the patient's primary language.

The University of California San Diego CME (UCSD CME) has developed several initiatives to incorporate cultural and linguistic competency into CME activities with patient care components. Compliance with AB1195 will be ensured by including cultural and linguistic elements into the educational planning and content of the program and/or by distributing cultural and linguistic resources to CME program attendees.

Enclosed in this package you will find a brief review of federal and state law regarding linguistic access and services along with a list of useful resources. For additional resources and a copy of AB1195, please visit our website at http://cme.ucsd.edu.

Brief Review of Federal and State Law
Regarding Linguistic Access and Services for Limited English Proficient Persons
Prepared for the UC CME Consortium
by the UC Office of General Counsel

I. Purpose.

This document is intended to satisfy the requirements set forth in California Business and Professions code 2190.1. California law requires physicians to obtain training in cultural and linguistic competency as part of their continuing medical education and professional development programs. This document and the accompanying attachments are intended to provide physicians with an overview of federal and state laws regarding linguistic access and services for limited English proficient ("LEP") persons. The document is not comprehensive and there may be additional federal and state laws governing the manner in which physicians and healthcare providers render services for disabled, hearing impaired or other protected categories. We recommend that physicians review the CMA California Physician's Legal Handbook for a comprehensive review of laws affecting a physician's medical practice in California.

II. Federal Law – Federal Civil Rights Act of 1964, Executive Order 13166, August 11, 2000, and Department of Health and Human Services ("HHS") Regulations and LEP Guidance.

The Federal Civil Rights Act of 1964, as amended, and HHS regulations require recipients of federal financial assistance to take reasonable steps to ensure that LEP persons have meaningful access to federally funded programs and services.
HHS recently issued revised guidance documents for Recipients to ensure that they understand their obligations to provide language assistance services to LEP persons. A copy of HHS's summary document entitled "Guidance for Federal Financial Assistance Recipients Regarding Title VI and the Prohibition Against National Origin Discrimination Affecting Limited English Proficient Persons – Summary" is attached for your review. Additional in-depth guidance is available at HHS's website at: http://www.hhs.gov/ocr/lep/ .

As noted above, Recipients generally must provide meaningful access to their programs and services for LEP persons. The rule, however, is a flexible one and HHS recognizes that "reasonable steps" may differ depending on the Recipient's size and scope of services. HHS advised that Recipients, in designing an LEP program, should conduct an individualized assessment balancing four factors, including: (i) the number or proportion of LEP persons eligible to be served or by the Recipient; (ii) the frequency with which LEP individuals come into contact with the Recipient's program; (iii) the nature and importance of the program, activity or service provided by the Recipient; and (iv) the resources available to the Recipient and the costs of interpreting and translation services.

Based on the Recipient's analysis, the Recipient should then design an LEP plan based on five recommended steps, including: (i) identifying LEP individuals who may need assistance; (ii) identifying language assistance measures; (iii) training staff; (iv) providing notice to LEP persons; and (v) monitoring and updating the LEP plan.

A Recipient's LEP plan likely will include translating vital documents and providing either on-site interpreters or telephone interpreter services, or using shared interpreting services with other Recipients. Recipients may take other reasonable steps, such as hiring bilingual staff who are competent in the skills required for medical translation, hiring staff interpreters, or contracting with outside public or private agencies that provide interpreter services.

III. California Law – Dymally-Alatorre Bilingual Services Act.

The California legislature enacted the California's Dymally-Alatorre Bilingual Services Act (Govt. Code 7290 *et seq.*) in order to ensure that California residents would appropriately receive services from public agencies regardless of the person's English language skills. California Government Code section 7291 recites this legislative intent as follows:

The Act generally requires state and local public agencies to provide interpreter and written document translation services in a manner that will ensure that LEP individuals have access to important government services. Agencies may employ bilingual staff, and translate documents into additional languages representing the clientele served by the agency. Public agencies also must conduct a needs assessment survey every two years documenting the items listed in Government Code section 7299.4, and develop an implementation plan every year that documents compliance with the Act. A copy of this law may be found at the following url: http://www.spb.ca.gov/bilingual/dymallyact.htm

University of California (UCCME)
Cultural and Linguistic Competency Resources
June 2006

A) Major Resources

1. University of California-Center for the Health Professions
http://futurehealth.ucsf.edu/TheNetwork/Default.aspx?tabid=387

2. Kaiser Permanente National Diversity Department
http://kphci.org/resources/links.html

3. The Office of Minority Health
http://www.omhrc.gov

4. California Academy of Family Physicians
http://www.familydocs.org/multicultural_health.php

5. Institute for Medical Quality
www.imq.org

6. On-line dictionary providing translations into 25 different languages
http://www.ectaco.com/English-Multilanguage-Dictionary/

7. Foreign Language Assessment Guide (F.L.A.G.), Produced by Medi-Flag Corporation
www.medi-flag.com

B) Hospital Care

1. National Association of Public Hospitals and Health Systems. "Serving Diverse Communities in Safety Net Hospitals and Health Systems," The Safety Net 2003; 17(3): Fall.
http://www.naph.org/Template.cfm?Section=The_Safety_Net_Archive&template=/ContentManagement/ContentDisplay.cfm&ContentID=3407

2. Andrulis DP. "Study of How Urban Hospitals Address Sociocultural Barriers to Health Care Access":
http://www.rwjf.org/portfolios/resources/grantsreport.jsp?filename=023299s.htm&iaid=133

C) Ambulatory Care

1. Center for the Health Care Professions- Towards Culturally Competent Care: Toolbox for Teaching Communication Strategies
http://futurehealth.ucsf.edu/TheNetwork/Default.aspx?tabid=290

2. National Center for Cultural Competence, Georgetown University. "Self-Assessment Checklist for Personnel Providing Primary Health Care Services"
http://gucchd.georgetown.edu/nccc/documents/Checklist%20PHC.pdf

3. National Initiative for Children's Healthcare Quality (NICHQ), Improving Cultural Competency in Children's Health Care: Expanding Perspectives
http://www.nichq.org/NR/rdonlyres/5B534B7B-0C38-4ACD-8996-EBB0C4CB2245/0/NICHQ_CulturalCompetencyFINAL.pdf

4. "Cultural Positivity – Culturally Competent Care For Diverse Populations"
http://www.gvhc.org/

D) Managed Care

1. "National Standards For Culturally And Linguistically Appropriate Services In Health Care Executive Summary"
http://www.omhrc.gov/assets/pdf/checked/executive.pdf

2. America's Health Insurance Plans (AHIP), Center for Policy and Research. "Innovations in Medicaid Managed Care," March, 2005.
http://www.ahip.org/content/default.aspx?docid=8414

E) Caring for Individuals with Limited English Proficiency

1. Center for the Health Professions-Common Sentences in Multiple Languages (ICE) Tool for Office Staff
http://futurehealth.ucsf.edu/TheNetwork/Portals/3/CommonSentences.pdf

2. National Council on Interpreting in Health Care
http://www.ncihc.org

3. Addressing Language Access in Your Practice Toolkit, California Academy of Family Physicians
http://www.familydocs.org/multicultural_health.php

4. Hablamos Juntos: Improving Patient-Provider Communication for Latinos
http://www.hablamosjuntos.org

5. Process of Inquiry: Communicating in a Multicultural Environment, Georgetown University National Center for Cultural Competence
http://www.nccccurricula.info/

6. Cross-Cultural Communication in Health Care: Building Organizational Capacity
http://www.hrsa.gov/reimbursement/broadcast/default.htm

F) Health Literacy

1. AMA/AMA Foundation's Health Literacy toolkits, videos, partnerships
http://www.ama-assn.org/ama/pub/category/8115.html

2. Weiss BD. Health Literacy: A Manual for Clinicians Chicago: American Medical Association Foundation, 2003

3. Schwartzberg, JG, VanGeest JB, Wang CC: Understanding Health Literacy: Implications for Medicine and Public Health. Chicago, IL: American Medical Association Pres., 2004

G) Movies, Videos, and CD-ROM Resources

1. Alexander M. Cinemeducation: An Innovative Approach to Teaching Multi-Cultural Diversity in Medicine. Annals of Behavioral Science and Medical Education 1995; 2(1):23-28.

2. Communicating Effectively Through an Interpreter (1998) (Available from the Cross Cultural Health Care Program, 270 South Hanford Street, Suite 100, Seattle, Washington 98134; Phone (206)-860-0329; Website www.xculture.org).

3. The Bilingual Medical Interview I (1987) and The Bilingual Medical Interview II: The Geriatric Interview, Section of General Internal Medicine, Boston City Hospital, in collaboration with the Department of Interpreter Services and the Boston Area Health Education Center (Available from the BAHEC, 818 Harrison Ave., Boston, MA 02118; Phone (617) 534-5258).

4. The Kaiser Permanente/California Endowment Clinical Cultural Competency Video Series. In 2000, Kaiser Permanente, with funding from The California Endowment, embarked on a project to create "trigger" videos as teaching tools for training healthcare professionals in cultural competence. These now completed videos comprise three sets, each with accompanying facilitator's guide and contextual materials. Each set costs $35.00 or $105 for all 20. The scenarios are from eight to fourteen minutes long.

5. Quality Care for Diverse Populations. Video/CD-ROM/Facilitator's Guide, Contributors: K. Bullock, L.G. Epstein, E.L. Lewis, R.C. Like, J.E. South Paul, C. Stroebel, et al) This educational program includes five video vignettes depicting simulated physician-patient visits in an office setting as a means to explore ethnic and sociocultural issues found in today's diverse health care environment. Produced by the American Academy of Family Physicians (AAFP), with partial funding by the Bureau of Primary Health Care, Health Resources and Services Administration, June 2002. (Available from the American Academy of Family Physicians, AAFP Order Dept., 11400 Tomahawk Creek Parkway, Leawood, KS 66211; Phone (800) 944-0000; Fax (913) 906-6075; http://www.aafp.org/x13887.xml).

6. Community Voices: Exploring Cross-Cultural Care Through Cancer. Video and Facilitator's Guide by Jennie Greene, MS & Kim Newell, MD (Available from the Harvard Center for Cancer Prevention, Harvard School of Public Health, 665 Huntington Avenue, Bldg 2, Rm 105, Boston, MA 02115; Phone (617) 432-0038; Fax: (617)-432-1722; hccp@hsph.harvard.edu , or Fanlight Productions, www.fanlight.com).

7. Worlds Apart. A Four-Part Series on Cross-Cultural Healthcare. By Maren Grainger-Monsen, MD, and Julia Haslett, Stanford University, Center for Biomedical Ethics (available from Fanlight Productions, www.fanlight.com)

8. The Angry Heart: The Impact of Racism on Heart Disease Among African-Americans, Jay Fedigan. (Available from Fanlight Productions, www.fanlight.com).

9. The Culture of Emotions: A Cultural Competence and Diversity Training Program . Harriet Koskoff, Producer/Co-Coordinator, 415 Noe Street, #5, San Francisco , CA 94114 ; Phone 415-864-0927; Fax 415-621-8969 (Available from Fanlight Productions, www.fanlight.com).

10. Ohio Department of Health and Medical College of Ohio. Cultural Competence in Breast Cancer Care (CD-ROM), 2000.

H) Continuing Education Programs

1. Office of Minority Health
A Family Physician's Guide to Culturally Competent Care
http://cccm.thinkculturalhealth.org

2. Quality Interactions: A Patient-Based Approach to Cross-Cultural Care
Manhattan Cross Cultural Group and Critical Measures
http://www.criticalmeasures.net/cross_cultural/elearning.htm

3. Delivering Culturally Effective Care for Patients with Diabetes
Medical Directions - The Virtual Lecture Hall and Department of Family Medicine, University of Arizona College of Medicine at the Arizona Health Sciences Center
http://www.vlh.com/shared/courses/course_info.cfm?courseno=1786

4. Communicating Through Health Care Interpreters
Medical Directions - The Virtual Lecture Hall and Rush University Medical Center
http://www.vlh.com/shared/courses/course_info.cfm?courseno=1705

5. Culture and Health Care: An E-Learning Course (based on Cultural Sensitivity: A Guidebook for Physicians and HealthCare) Doctors in Touch (DIT)
http://www.doctorsintouch.com/courses_for_CME_credit.htm

6. Quality Care for Diverse Populations. Video/CD-ROM/Facilitator's Guide, Contributors:
K. Bullock, L.G. Epstein, E.L. Lewis, R.C. Like, J.E. South Paul, C. Stroebel, et al) This educational program includes five video vignettes depicting simulated physician-patient visits in an office setting as a means to explore ethnic and sociocultural issues found in today's diverse health care environment. Produced by the American Academy of Family Physicians (AAFP), with partial funding by the Bureau of Primary Health Care, Health Resources and Services Administration, June 2002. (Available from the American Academy of Family Physicians, AAFP Order Dept., 11400 Tomahawk Creek Parkway, Leawood, KS 66211; Phone (800)-944-0000; Fax (913)-906-6075; http://www.aafp.org/x13887.xml).

7. Cultural Competency Challenge CD-ROM Educational Program (AAOS Product #02735). American Academy of Orthopaedic Surgeons, 6300 North River Road, Rosemont, IL 60018-4262 www.aaos.org/challenge).

8. Cross-Cultural Health Care: Case Studies
Pediatric Pulmonary Centers: A Collaborative Web Site of the MCH Training Network
http://ppc.mchtraining.net/custom_pages/national_ccce

9. Measuring Health Disparities, Interactive CD-ROM. John Lynch, PhD, and Sam Harper, PhD, McGill University. Produced by the Michigan Public Health Training Center (MPHTC)
http://measuringhealthdisparities.org

I) Recent Articles and References on Cultural and Linguistic Competency

1. Brach C, Fraser I, and Paez K. "Crossing the Language Chasm," Health Affairs 2005 (March); 24(2):424-434.

2. Betancourt, J.R., Green, A.R., Carillo, J.E. et al. (2005). Cultural competency and health care disparities: Key perspectives and trends. Health Affairs, 24(2), 499-505.

3. Brach, C., Fraser, I., Paez, K. (2005). Crossing the language chasm: An in-depth analysis of what language-assistance programs look like in practice. Health Affairs, 24(2), 424-434.

4. Betancourt J.R., Green A.R., Carrillo J.E., et al. (2003). Defining cultural competence: A practical framework for addressing racial/ethnic disparities in health and health care. Public Health Reports, 118(4), 293-302.

5. AB 801 Assembly Bill – Chaptered. Official California Legislative Information website. http://www.leginfo.ca.gov/pub/03-04/bill/asm/ab_0801-0850/ab_801_bill_20030925_chaptered.html (cited 7 Nov. 2005).

6. AB 1195 Assembly Bill – Chaptered. Official California Legislative Information website. http://www.leginfo.ca.gov/pub/bill/asm/ab_1151-1200/ab_1195_bill_20051004_chaptered.html (cited 7 Nov. 2005).

7. Youdelman M, Perkins J. "Providing Language Interpretation Services in Health Care Setting: Examples from the Field," National Health Law Program, May 2002

8. Youdelman M, Perkins J. "Providing Language Services in Small Health Care Provider Settings" Examples from the Field," National Health Law Program, April 2005
(The latter two reports can be obtained at www.cmwf.org or www.healthlaw.org).

Compiled by:
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NITRIC OXIDE SYMPOSIUM

Session Chair:
Filippo Drago, Ph.D.

EFFECTS OF NITRIC OXIDE SYNTHASE INHIBITION ON CILIARY BLOOD FLOW, AQUEOUS PRODUCTION AND INTRAOCULAR PRESSURE

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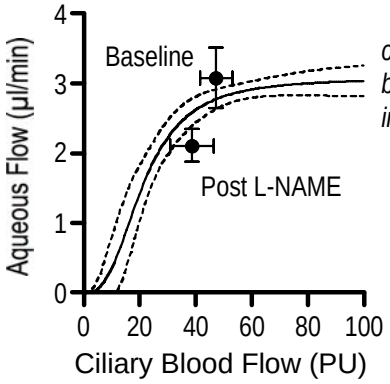
Purpose: Nitric oxide is a primary regulator of choroidal blood flow, and systemic inhibition of nitric oxide synthase with L-NAME causes choroidal vasoconstriction and a sustained decrease in IOP in rabbits (Exp Eye Res 69: 413-429, 1999). While the intial IOP drop is explained by expulsion of choroidal blood volume, the sustained ocular hypotension suggests an effect on aqueous dynamics. The present study tested the hypothesis that L-NAME decreases aqueous production by reducing ciliary blood flow below the level needed to sustain ciliary metabolism. Methods: Two protocols were performed in pentobarbital anesthetized rabbits. In the first protocol, mean arterial pressure (MAP) and IOP were measured by direct cannulation, and aqueous flow was measured by fluorophotometry, before and after L-NAME (5 mg/kg, iv, n=7). In the second protocol, ciliary blood flow was measured by laser Doppler flowmetry while MAP was varied mechanically over a wide range before and after L-NAME (5 mg/kg, iv, n=9). Results: L-NAME increased MAP (14% ± 2%), and decreased IOP (-24% ± 2%), aqueous flow (-25% ± 6%) and ciliary blood flow (-21% ± 9%). L-NAME also caused a marked downward shift in the ciliary pressure-flow relation.



control relationship between ciliary blood flow and aqueous production in the anesthetized rabbit.

Conclusions: L-NAME causes ciliary vasoconstriction and decreased aqueous production, suggesting that L-NAME's hypotensive effect is due to a blood-flow dependent decrease in aqueous production. However, assuming no uveoscleral outflow and constant episcleral venous pressure (8 mmHg) and outflow facility (0.27 µl/min/mmHg), the decrease in aqueous flow accounts for 66% of the drop in IOP, suggesting that L-NAME also facilitates aqueous outflow.

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NITRIC OXIDE AND CARBON MONOXIDE IN THE EYE: TWO FACES OF THE SAME COIN

C Bucolo and F Drago
Bausch & Lomb

Nitric oxide (NO) and carbonic monoxide (CO) are organic gases ubiquitously synthesized in mammalian tissues by enzymes that have constitutive and inducible forms. They are produced as metabolic end-products in specific cell life phases, and may act as neuronal messengers. Systems that generate NO resemble those generating CO, as both NO and CO share affinity for the heme molecule and activate guanylate cyclase and increase cGMP synthesis. Next, NO and CO might be possibly involved in apoptosis of neurons, even though their role is still partly unknown as they can either promote or counteract neuronal death depending on circumstances. Many works have highlighted the role of NO in a wide range of ocular diseases, but very few have done the same for CO. Hemin, a potent inducer of heme oxygenase-1 (HO-1), protects against LPS-induced uveitis in rats by down-regulating NO and pro-inflammatory cytokines expression. Thus, in ocular tissues CO generated from heme by heme oxygenase may regulate NO and exert cytoprotective properties. Induction of HO-1 by hemin has been found to prevent retinal cell death after ischemia provoked by ocular hypertension in rats. The LPS-induced expression of pro-inflammatory cytokines, in rat eye, is also inhibited by CO. Interestingly, drugs active as inhibitors of iNOS block CO-induced increases in cGMP in the retina. Drugs inhibiting NO formation by acting on iNOS activity have been found to potently reduce intraocular pressure. Studies from our lab showed that an increase of CO levels induced by hemin lower significantly the intraocular pressure (IOP) in rabbit. It is possible that hemin-derived CO reduces IOP in rabbits with ocular hypertension by suppressing iNOS-derived NO production, suggesting that NO and CO in the eye are interconnected. Therefore, hemin and more in general the HO-1 inducers (i.e. phenolic compounds) may offer a novel class of agents potentially effective in the modulation of IOP and retinal protection.

IN VIVO AND IN VITRO EVALUATION OF NITRIC OXIDE ACTIVITY IN THE EYE

G. Prasanna, S. Siagel, D. Gale, and A. Krauss
Pfizer Global R&D

Purpose: Nitric oxide (NO) is responsible for a variety of physiological functions throughout the body. In the eye, several tissues are capable of generating NO and utilizing it for regulation of IOP and ocular blood flow. Alterations of this pathway may play a role in the development of glaucoma. Due to the transient nature of NO, it is difficult to assess its levels in real time. Therefore, the purpose of this study was to: 1) evaluate a method of detecting NO levels in real time in ocular cells; 2) establish a method of detecting NO and cGMP (a marker of NO activity) in rabbit ocular tissue; and 3) assess the release kinetics of nitric oxide from NO Donors using these two methods.

Methods: In vitro: Human primary ocular cells were incubated with NO donors after loading with diaminofluorescein (DAF) and nitric oxide release was detected via fluorescence microscopy and quantified using ImagePro. In vivo: Sodium nitroprusside (SNP) a NO-donating compound was administered topically to the eyes of normotensive rabbits. NO levels were assessed in the aqueous humor and iris/ ciliary body (ICB) at various time points using a Nitrate/Nitrite Fluorometric assay kit. Levels of cGMP were also measured using an EIA kit.
Results: In vitro: SNP produced a time and concentration-dependent increase in fluorescence intensity in human primary ciliary smooth muscle cells. In vivo: NO was detected at low levels in the rabbit aqueous humor and ICB after topical administration of SNP. However, cGMP levels increased more dramatically compared to NO levels after topical dosing with the SNP, indicating that determination of cGMP levels may be more suitable for in vivo assessment of NO activity.

Conclusions: The in vitro cell based assay is an effective method of determining the real-time kinetics of NO release and assessment of its intracellular levels. The Nitrite/Nitrate fluorometric assay is capable of detecting NO in rabbit samples but the cGMP EIA kit provides a better window from control in a time course study. Therefore, both the in vitro and in vivo assays provide valuable information when studying nitric oxide release from NO donors in the eye.

Disclosures:
Employee of Pfizer Global R&D

INVOLVEMENT OF NEURONAL NITRIC OXIDE SYNTHASE IN RETINAL BLOOD FLOW AND CELLULAR FUNCTION

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The main objective was to determine if neuronal nitric oxide synthase (nNOS) plays a role in control of retinal blood flow and retinal cellular function. Retinal blood velocities in arteries, veins, and small vessels (<40 μm diameter) were determined by tracking 1 μm yellow-green fluorescent microspheres in anesthetized pigmented adult rats using a Scanning Laser Ophthalmoscope (SLO). Directional flow of fluorescent microspheres and vessel characterization were determined based on examination of infrared reflectance (IR) and fluorescein angiogram (FA) images. Post-treatment velocity measurements were obtained every 15 minutes for ~2 hours. In separate experiments, retinal cellular function was assessed by dark-adapted corneal single- and paired-flash electroretinogram (ERG). The paired-flash ERG technique allows determination of the full time course of the rod response to a test flash of arbitrary intensity in vivo. The NO levels were modulated by one 3 μl intravitreal injection of either nitric oxide synthase (NOS) inhibitors, NG-nitro-L- arginine methyl ester (L-NAME, 2.5 mM vit. conc, nonselective inhibitor) and 1-(2-trifluoromethylphenyl) imidazole (TRIM, 2 mM vit. conc, nNOS inhibitor) or a NO donor, S-nitroso-N-acetylpenicilliamin (SNAP, 2 mM vit. conc). Intraocular pressure (IOP) was measured before and after the injection. There were no significant changes in IOP (average of 14 mmHg) before and after the injection indicated that the pressure in the eye remained uniform and did not have a direct effect on retinal cellular activity or blood velocity. TRIM treatment decreased arterial velocity by ~18%, venous velocity by ~15% and small vessel velocity by ~5%. After L-NAME treatment, arterial velocity decreased significantly by ~17% and venous velocity decreased by ~32%. SNAP treatment also increased arterial velocity by ~28% and venous velocity by ~13%. Single flash a- and b-wave amplitudes increased ~5% and ~20%, respectively, after the TRIM treatment. Time courses obtained through the paired-flash recordings after the TRIM injection were also altered. At a brighter test flash, the recovery of time course was slower compared to the pre- TRIM injection time course. These results suggested that nNOS may be involved in both retinal signal processing and retinal blood flow control.

Support: Whitaker Foundation

OCULAR SURFACE PHARMACOLOGY

Session Chairs:
Michael Stern, Ph.D. and
Stephen Pflugfelder, M.D.

USE OF COMPATIBLE SOLUTES TO PROTECT THE OCULAR SURFACE FROM HYPEROSMOLAR STRESS

Peter A Simmons[1], Joseph G Vehige[1], Joan-En Chang-Lin[1], Stephen C Pflugfelder[2]
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Purpose: Dry eye disease is characterized in most instances by a hyperosmolar tear fluid, which has been shown to induce pro-inflammatory changes in the ocular surface. Compatible solutes are a specific class of small, non-ionic organic compounds used by numerous tissues and organs, as well as by many plant and animal species, to physically protect cells from the dehydrating effects of hyperosmolar environments. We have explored the potential of utilizing such compatible solutes in a topical ophthalmic solution to protect ocular cells in dry eye conditions.

Methods: Hyperosmolar conditions were produced in vitro to simulate ocular surface cell osmotic stress. Candidate compatible solutes including several amino acids and small polyols were evaluated for osmoprotective effects using a primary culture of rabbit corneal epithelial cells grown under air-interface conditions to produce a multiple-layered organotypic test system. Transepithelial electrical resistance (TEER) was used as a measure of overall epithelial health and viability. Additionally, primary human corneal cells grown in monolayer culture were used to assess activation of MAP kinases (cellular stress markers) with or without the presence of compatible solutes. Ratios of phophorylated (activated) to total JNK, p38, and ERK were measured in cell lysates with standard ELISA methods or with Beadlyte multi-assay techniques.

Results: Under hyperosmolar conditions, several combinations of compatible solutes, including glycerol, l-carnitine, betaine, and erythritol, maintained the TEER value of the cultures at levels similar to that of cultures left in isotonic conditions. Similarly, activation of MAP kinases due to hyperosmolar stress was reduced in the presence of compatible solutes, particularly carnitine and betaine. Both types of protective effects were specific to certain compatible solutes and were concentration-dependent.

Conclusions: In model systems of corneal epithelial cells, compatible solutes have been shown to protect against the damaging effects of a hyperosmolar environment. This demonstrates potential for application in artificial tear formulations.

Disclosures:
Peter Simmons is an employee of Allergan, LLC, which is a manufacturer of products related to the research reported in this presentation.

IFN-γ IS REQUIRED FOR DISEASE PATHOLOGY IN A T_H2 MOUSE MODEL OF ALLERGIC CONJUNCTIVITIS

M. E. Stern[1], S. C. Pflugfelder[2], K. F. Siemasko[1], J. Gao[1], V. L. Calder[3], J. Y. Niederkorn[4].
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Purpose: Dry eye and allergic conjunctivitis are ocular inflammatory diseases that are mediated by $CD4^+$ T effector cells. IFN-γ secreting T_H1 cells induce the disease process in dry eye. Allergic conjunctivitis has been classically defined as a T_H2-mediated pathology due to the presence of the T_H2 cytokines IL-4 and IL-13. The role of IFN-γ on ocular inflammation in a mouse model of allergic conjunctivitis (classic T_H2 animal model) alone or combined with a dry, desiccating environment (classic T_H1 animal model) were evaluated in BALB/c WT mice and IFN-γ knockout mice.

Methods: BALB/c WT mice or IFN-γ knockout (KO) mice were sensitized with 20 μg short ragweed (SRW) in alum in the left hind footpad. Beginning ten days later, SRW topical secondary challenges to the ocular surface were applied on days 10-16 in the absence or presence of scopolamine 3X/day in the presence of a low humidity chamber and constant air flow to create a dry, desiccating environment (DS).

Results: SRW sensitized and challenged mice exposed to a desiccating environment (T_H1-type stimulus) had a significant increase in the number of inflammatory cells infiltrating the conjunctiva as compared to SRW sensitization and challenge alone. SRW sensitized and challenged IFN-γ KO mice had a significantly decreased inflammatory cell infiltration into the conjunctiva as compared to the SRW challenged WT group (77% decrease in eosinophils; 35 % decrease in neutrophils). IFN-γ knockout mice had reduced VCAM-1 endothelial cell expression.

Conclusions: Conjunctival cellular infiltration of SRW sensitized and challenged animals in the presence of a dry, desiccating environment were significantly increased providing evidence that the presence of T_H1 cytokine mediated disease can further intensify a T_H2-like disease pathology. IFN-γ is unexpectedly required for the immunopathology of allergic conjunctivitis disease. The T_H1 cytokine IFN-γ acts as an endothelium gatekeeper by inducing endothelial expression of vascular adhesion molecule-1 necessary for inflammatory conjunctival cellular infiltration.

Disclosures:
Michael Stern is an employee of Allergan

ROLE OF T REGULATORY CELLS IN AUTOIMMUNE LACRIMAL KERATOCONJUNCTIVITIS

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[1]*Department of Ophthalmology, Baylor College of Medicine*

Purpose: To determine the role of T regulatory cells in the induction of experimental autoimmune lacrimal keratoconjunctivitis.

Methods: BALB/c or C57BL/6 wild type (WT) mice were exposed to a desiccating environmental stress (DS) for 5 days as previously described (Dursun et al., 2002). Magnetic microbead isolation kits were used to isolate CD4+ and CD4+CD25+ T regulatory cells from mouse spleen and CLN. FoxP3+ intracellular expression was confirmed by flow cytometry. One mouse equivalent of CD4+ T cells (2 X 106 cells) was IP injected into syngeneic T cell deficient mice or BALB/c WT mice kept under non-stressed (NS) conditions. For reconstitution experiments, equivalent numbers of T regulatory cells were adoptively transferred.

Results: DS donor CD4+ T cells adoptively transferred to athymic recipients resulted in significantly diminished tear production, conjunctival goblet cell loss and cellular infiltration into the LFU. These findings were not observed follwing transfer of CD4+ T-cells from NS mice. Adoptive transfer of DS CD4+ T cells into euthymic mice produced minimal disease pathology, implying an inhibitory effect of T regulatory cells in the WT recipient. Athymic mice reconstituted with CD4+CD25+FoxP3+ T regulatory cells on the same day they received DS CD4+ T cells resisted inflammation with a 60% reduction in mononuclear cells and a 97% decrease in neutrophils that infiltrated the conjunctiva.

Conclusions: A CD4+ T cell mediated inflammatory event is activated in response to ocular surface desiccation. Reconstitution of nude mice with activated CD4+ cells and T regulatory cells abrogates the development of autoimmune lacrimal keratoconjunctivitis.

Supported by NIH Grant EY11915 (SCP) and Allergan, Inc.

KEYNOTE PRESENTATION

Rafat Ansari, Ph.D.

NON-INVASIVE AND EARLY DETECTION OF OCULAR AND SYSTEMIC DISEASES USING "EYE AS A WINDOW TO THE BODY"

R. Ansari
Vision Research and Human Health Diagnostics Laboratory

As a "window to the body", the eye offers the opportunity to use light in various forms to detect ocular and systemic abnormalities long before clinical symptoms appear and help develop preventative/therapeutic countermeasures early. The effects of space travel on human body are similar to those of normal aging. For example, radiation exposure in space could lead to formation of cataracts. The zero-gravity environment causes fluid shifts to the upper extremities of the body leading to osteoporosis. Here on Earth, cataract, age-related macular degeneration (AMD), diabetic retinopathy (DR), and glaucoma are major eye diseases and are expected to double in next two decades. To detect, prevent, and treat untoward effects of prolonged space travel in real-time requires the development of non-invasive diagnostic technologies that are compact and powerful. Results will be presented on the development of novel fiber-optic sensors, flight experiments, laboratory and clinical experiments to evaluate the ocular tissues in health, aging, and disease employing the techniques of dynamic light scattering (cataract, uveitis, Alzheimer's, glaucoma, DR, radiation damage, refractive surgery outcomes), auto-fluorescence (aging, DR), laser-Doppler flowmetry (choroidal blood flow), Raman spectroscopy (AMD), polarimetry (diabetes), and retinal oximetry (occult blood loss). The non-invasive feature of these technologies integrated in a head-mounted/goggles-like device will permit frequent repetition of tests, enabling evaluation of the results to therapy that may ultimately be useful in various celestial and terrestrial telemedicine applications.

NEW TECHNIQUES FOR OCULAR DRUG DEVELOPMENT

Session Chairs:
Makoto Aihara, M.D., Ph.D. and
Iok-Hou Pang, Ph.D.

BIOINFORMATIC RESOURCES APPLIED TO INHERITED EYE DISEASES

T Braun, T Scheetz, T Casavant, V Sheffield, E Stone, A Clark
University of Iowa

Purpose: The pursuit of the genetic causes of inherited eye diseases has led to the development of genomic, proteomic, and bioinformatic resources.

Methods: The University of Iowa, in collaboration with Alcon Research, has developed multiple bioinformatics and genomic resources utilizing diverse high-throughput technologies to explore the genetics of inherited eye diseases. These include expression profiling in ocular tissues using expression arrays; characterization of human trabecular meshwork proteome with two-dimensional PAGE; and expression quantitative trait loci (eQTL) mapping in rat eyes.

Results: 1) The ocular tissue database (OTDB) is a collection of expression profiles of genes from 12 human ocular tissues using Affymetrix Plus2 expression arrays. A web-accessible interface has been developed to enable queries based on tissue, gene names, or probe names. 2) A proteomic survey of a TM cell line has been implemented and deployed as a resource available to the community. 3) Gene expression regulation has been mapped (expression quantitative trait loci -- eQTL) in rat eyes using 120 in-bred rats, Affymetrix expression arrays, and 400 genetic markers.

Conclusions: Utilizing multiple data resources for target identification in ocular disorders has facilitated the development of a bioinformatics framework for integrating disparate data sources. The Candidate List Prioritization Heuristic (CLiPH) is a bioinformatics framework for integrating disparate data sources for the purpose of prioritizing candidate disease genes for mutation screening and therapeutic target identification.

USING GENOMICS TO IDENTIFY NEW GLAUCOMA THERAPEUTIC TARGETS

AF Clark. Presented by **Iok-Hou Pang**
Alcon Research, Ltd.

Purpose: Intraocular pressure (IOP) is a major causative risk factor for the development and progression of glaucoma. IOP lowering therapeutics are the current standard of care for the treatment of glaucoma; however, these therapies do not address the underlying cause of elevated IOP. We are using genomics techniques to identify molecular pathogenic pathways responsible for glaucomatous IOP elevation in order to discover new disease modifying therapeutic agents.

Methods: RNA was isolated from trabecular meshwork (TM) tissue and cultured TM cells obtained from normal and glaucomatous donor eyes. Gene expression was analyzed using Affymetrix gene chips and QRT-PCR to identify glaucoma associated differentially expressed genes. Perfusion organ cultured human eyes and adenovirus transgene transduction of rodent eyes were used to determine the effects of target genes on IOP.

Results: Three new glaucoma pathogenic pathways were identified. (1) The expression of sFRP1 mRNA and protein was elevated in glaucomatous TM cells. sFRP1 is an inhibitor of the WNT signaling pathway, and the trabecular meshwork has a functional WNT signaling pathway that regulates IOP. Recombinant sFRP1 added to perfusion cultured human eyes elevated IOP, and AdV.sFRP1 transduction of mouse eyes also elevated IOP. (2) The expression of SAA2 mRNA and protein was increased in glaucomatous TM cells and tissues, and SAA2 protein was elevated in glaucomatous aqueous humor samples. Recombinant SAA2 added to perfusion organ cultured eyes elevated IOP, and AdV.SAA2 transduction of rat and mouse eyes elevated IOP. (3) The expression of Gremlin mRNA and protein was elevated glaucomatous TM cells. Gremlin is an antagonist of BMP signaling, and we previously demonstrated that TM cells express BMPs and BMP receptors. BMP4 blocked TGFbeta2 induction of TM cell extracellular matrix, and Gremlin reversed this effect. Gremlin added to perfusion cultured human eyes elevated IOP.

Conclusion: We have identified new glaucoma pathogenic pathways that are associated with elevated IOP. Regulation of these new pathways may lead to new disease intervening therapeutic agents for the treatment of glaucoma.

Disclosures:
Employee, Alcon Research, Ltd.

MULLER GLIA ARE STEM CELLS IN ADULT MAMMALIAN RETINA

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University of Nebraska Medical Center

The retina in adult mammals, unlike those in lower vertebrates such as fish and amphibians, is not known to support neurogenesis. However, when injured, the adult mammalian retina displays neurogenic changes, raising the possibility that neurogenic potential may be evolutionarily conserved and could be exploited for regenerative therapy. We demonstrate that Müller cells, when retrospectively enriched from the normal retina, like their radial glial counterparts in the central nervous system (CNS), display cardinal features of neural stem cells (NSCs), i.e., they self renew and generate all three basic cell types of the CNS. In addition, they possess the potential to generate retinal neurons, both in vitro and in vivo. By transplanting prospectively enriched injury-activated Müller cells into normal eye, a direct evidence is provided that Müller cells have neurogenic potential and can generate retinal neurons, confirming a hypothesis, first proposed in lower vertebrates. This potential is likely due to the NSC nature of Müller cells that remains dormant under the constraint of non-neurogenic environment of the adult normal retina. Additionally, it is demonstrated that the mechanism of activating the dormant stem cell properties in Müller cells involves Wnt and Notch signaling, two known regulators of stem cells in general. Together, these results identify Müller cells as latent NSCs in the mammalian retina and hence, may serve as a potential target for cellular manipulation for treating retinal degeneration.

AQUEOUS FLOW MEASURED BY FLUOROPHOTOMETRY IN THE MOUSE

CB Toris, S Fan, TV Johnson, B Ishimoto
University of Nebraska Medical Center

Purpose: This study evaluates a noninvasive fluorophotometric method to measure aqueous flow in the mouse.

Methods: CD1 mice were divided into three groups, ketamine/xylazine anesthesia (100-200 mg/kg and 5-16 mg/kg, respectively, n=18), tribromoethanol anesthesia (Avertin, 0.4 mg/gm body weight, n=6), Avertin anesthesia plus topical timolol (one 10 µl drop of 0.5% dosed one hour prior to anesthesia, n=7). Ninety minutes following topical application of one 10 µl drop of 0.1% fluorescein, animals were anesthetized and placed on a heated platform in front of a modified Fluorotron Master fluorophotometer. Scans were collected at 15 minute intervals for 1.5 to 2 hours. Another group of mice underwent an invasive, needle method to measure aqueous flow. With the animal anesthetized with ketamine/xylazine, two glass needles were inserted into the eye. FITC dextran (70 kD, 2%) was infused into the anterior chamber at 3 µl /min through one needle and removed at the same rate through another needle. After 20 minutes, the concentration of fluorescein in the infusion and withdrawal tubes were measured with a spectrofluorometer. The infusion rate and the amount of dilution were used to calculate aqueous flow as described by Aihara (IOVS, 2003).

Results: Under ketamine/xylazine anesthesia, aqueous flow by fluorophotometry was 0.09±0.01 µl/min. The modified fluorophotometer measured separate and distinct fluorescein concentrations in the cornea and anterior chamber. The slopes of the two decay curves were identical (-0.005). Aqueous flow in a dead mouse was zero. By the needle method aqueous flow was 0.38±0.07 µl/min. Aqueous flow in a subset of animals (n=8) measured with both methods was 0.09±0.03 by fluorophotometry and 0.28±0.07 µl/min by needle method (p=0.06). Under Avertin anesthesia, aqueous flow by fluorophotometry was 0.20±0.03 µl/min. This was reduced to 0.07±0.01 µl/min (p=0.001) with timolol treatment. Aqueous flow with ketamine/xylazine was significantly lower (p=0.001) than with Avertin anesthesia.

Conclusion: Aqueous flow in the mouse measured by fluorophotometry was lower than by the needle method. The needle method is technically more difficult with greater variability than the fluorophotometry method. The fluorophotometric method detected a significant reduction in aqueous flow following administration of timolol. Type of anesthesia did affect aqueous flow. The modified fluorophotometer may be useful for the study of aqueous flow in mice.

Supported by NIH grant EY016902 (BI)

OCULAR DRUG DELIVERY

Session Chair:
Peter Kador, Ph.D.

DURASITE AS A DRUG DELIVERY PLATFORM

M. Friedlaender, M.D., L. Bowman, Ph.D., and E. Si, Ph.D.
Scripps Clinic Research Foundation

DuraSite® (InSite Vision, Alameda, CA) is a proprietary drug delivery vehicle that stabilizes small molecules in a polymeric mucoadhesive matrix. The DuraSite solution of 1% azithromycin (AzaSite™, InSite Vision, Alameda, CA) was developed and recently evaluated in clinical trials. The physical properties of this solution were more robust than aqueous azithromycin and favorable for ophthalmic dosing.

The topical solution is a gel forming drop. This extends the residence time of azithromycin relative to conventional eye drops in the conjunctiva for up to several hours and permits the accumulation of a high concentration of antibiotic in the conjunctiva and on the ocular surface. Modeling of the concentrations achieved based on once-a-day topical dosing indicated peak and trough concentrations of 200 and 40µg/g, respectively. Unlike formulary preparations of azithromycin in water, AzaSite is stable for 9-12 months at room temperature.

Azithromycin is derived from the macrolide erythromycin. Classified as an azalide, azithromycin differs from erythromycin by having a 15 member ring with an aza- methyl-substituted nitrogen in the aglycone ring. In oral and intravenous delivery forms, azithromycin has higher tissue penetration, lower serum levels and a longer half-life than erythromycin. (Neu HC, 1991; Bryskier A and Labro MT, 1994) An azithromycin-based eye drop therapy for ocular infections was never developed before, possibly due to its poor solubility and stability in water. In ophthalmology, the use of azithromycin is limited to the treatment of trachoma by oral dosages. (West S, 1999)

In order to exploit the properties of azithromycin and expand the choices that physicians have for anti-infective treatment of ocular surface infections, an aqueous formulation was developed and evaluated in phase 3 clinical trials. The results from these studies show that AzaSite is safe and efficacious in treating acute bacterial conjunctivitis. The dosing regimen comprised of 2 drops on days 1-2 and a single drop on days 3-5. This represents a significant reduction in dosing as compared to conventional drops that require anywhere from 3-8 doses per day.

Disclosures:
Dr. Friedlaender is a Director of InSite Vision.

BACK-OF-THE-EYE EXPOSURE FOLLOWING TOPICAL ADMINISTRATION – INSIGHT INTO OCULAR TRANSPORT

Luis Dellamary+, Boris Klebansky*, Xinshan Kang*, Richard Fine*, Rich Soll+, Glenn Noronha+, Michael Martin+
*+TargeGen, Inc. and * BioPredict, Inc.*

Purpose: To investigate the dependence of physicochemical properties of drug substances on back-of-the-eye tissue exposure following topical instillation.

Methods: Sixteen amine-based drug substances across multiple structural classes were formulated and topically administered to C57/Bl6 mice. All drug substances were formulated at 10 mg/mL in a 1%HPMC/0.2%tyloxapol vehicle; a single 10 µL dose was administered. The resulting tissue concentrations were determined at 2 hours post administration via LC/MS/MS. Quantitative structure activity relationships (QSAR) were established for sclera/choroid tissue concentrations following topical instillation.

Results: Regression analysis suggests that back-of-the-eye tissue exposure best correlates with physicochemical parameters that describe molecular size such as the Van der Waals volume and PSA and with parameters that describe aqueous solubility such as logD.

Conclusions: For the 16 anime-based compounds employed in this investigation, molecular diffusion and solubility appear to be the main driving forces in the delivery to the back of the eye following topical administration.

Disclosures:
Employee of TargeGen, Inc

OCULAR DISPOSITION OF NOVEL JAK2/VEGF INHIBITORS FOLLOWING TOPICAL INSTILLATION

A. Kousba, J. Yu, L. Dellamary, R. White, S. Hu, A. Tabak, J. Cao, C. Ching Mak, J. Renick, A. McPherson, B. Zeng, V. Pathak, G. Ibanez, S. Stoughton, T. Olafson, R. Soll, J. Doukas, J. Hood, G. Noronha, M. Martin
TargeGen, Inc.

Purpose: To evaluate the disposition of novel, selective JAK2/VEGF inhibitors in mouse eye tissues following topical instillation and to assess their binding affinity in human eye retina and choroid tissues and in melanin.

Methods: The relative distribution of novel JAK2/VEGF inhibitors in BALB/c mouse eyes was evaluated following topical application of 0.1 to 1% doses. The concentration-time course to 7 days post-dose for the individual ocular tissues was employed for disposition assessments. Human eyes (donors aged 40-70 years) and melanin from Sepia officinalis were utilized to determine the compound binding affinity in vitro by equilibrium dialysis and the estimated concentrations of each compound were utilized for assessing the compound binding.

Results: The selected compounds achieved significant concentrations in the back of the eye which persist up to 7 days following single topical instillation. The compound showed high melanin and choroid binding affinity (95 to 99%) and low to moderate binding affinity in retina (52 to 81%).

Conclusions: The prolonged back of the eye tissues exposures following single topical installation of the selected compounds may be attributed to high melanin binding resulting in a choroidal depot.

Disclosures:
I am an employee at the company

AQUEOUS AND VITREOUS CONCENTRATION FOLLOWING TOPICAL ADMINISTRATION OF 1% VORICONAZOLE IN HUMANS

William F Mieler, G. Atma Vemulakonda, MD1†, Seenu M. Hariprasad, MD3‡,, Randall A. Prince, PharmD5 , Gaurav K. Shah, MD3, and Russell N. Van Gelder, MD, PhD1,2,3
University of Chicago

Objective: To determine the penetration of 1% voriconazole solution into the aqueous and vitreous cavities following topical administration.

Methods: A prospective, non-randomized clinical study of 13 phakic patients who underwent elective pars plana vitrectomy surgery for non-inflammatory indications. Samples were obtained and analyzed after topical administration of voriconazole 1% every two hours for 24 hours prior to surgery. Drug concentration quantitation was performed using high performance liquid chromatography (HPLC).

Results: Mean sampling time after topical administration of the final voriconazole was 24 ± 14 minutes. Mean voriconazole concentrations in aqueous and vitreous were 6.49 ± 3.04 µg/ml and 0.16 ± 0.082 µg/ml, respectively. Aqueous concentrations exceeded inhibitory MIC90 levels for a wide spectrum of fungi and mold including Aspergillus, Fusarium, and Candida species. Vitreous concentrations of voriconazole exceeded the MIC90 for Candida albicans.

Conclusions: Topically administered voriconazole achieves therapeutic concentrations in the aqueous of the non-inflamed human eye for many fungi and molds, and achieves therapeutic levels in the vitreous for Candida. With its broad spectrum coverage, high potency against organisms of concern, good tolerability, and intraocular penetration with topical administration, topical voriconazole may be a useful agent for the management of fungal keratitis and prophylaxis against the development of fungal endophthalmitis.

PHARMACOLOGY OF MYOPIA

Session Chairs:
Christine Wildsoet, Ph.D. and
Frank K. Shih, M.D.

ROLE OF VISION IN THE HOMEOSTATIC CONTROL OF EYE GROWTH AND ITS RELATION TO MYOPIA

Josh Wallman
City College of New York

Experimental studies in animals over the past few decades have shown that eye growth is strongly influenced by visual experience. Specifically, it is now clear that the eyes of young animals grow toward emmetropia by a visually guided homeostatic mechanism. This has been shown most convincingly by experiments in which animals compensate for myopia or hyperopia imposed by spectacle lenses, thereby restoring emmetropia. This compensation, which occurs in birds and mammals, including primates, is accomplished by altering both the rate of elongation of the eye and the thickness of the choroid. Evidence argues that the visual system can discern whether the defocus is myopic or hyperopic, probably by making use of optical aberrations in the eye, such as longitudinal chromatic aberration or spherical aberration.

It seems probable that myopia is a manifestation of a misdirection of this visually guided homeostatic growth control, rather than being the result of a genetic or anatomical defect. The challenge is to understand what causes this misdirection to take place. Recent findings suggest that we have overlooked the importance of the spatial and temporal patterns of myopic and hyperopic defocus experienced in daily life as possible factors in the etiology of myopia.

RETINAL SIGNALING IN THE CONTROL OF EYE GROWTH AND MYOPIA

William K. Stell
University of Calgary Faculty of Medicine

The retina analyzes visual images by partitioning image information into separate channels, representing (e.g.) differences or changes in space and time, wavelength and contrast. To know how the retina does this, and so to understand what the eye tells the brain, it is necessary to know which retinal neurons perform these tasks – to associate specific neurons with specific functions. This talk is concerned particularly with the functions of amacrine cells, a diverse and complex family of local-circuit retinal neurons that interact with bipolar and ganglion cells to fine-tune visual output to the brain.

One important function of the retina in general, and of amacrine cells in particular, is to achieve emmetropia (the matching of eye length to focusing power). This is accomplished by visual regulation of eye growth, using image quality as a feedback control signal. In animal models, including the young chickens used in our studies, deprivation of form vision or imposition of refractive error – with diffusers or lenses, respectively – causes rapid and marked changes in ocular growth and refraction. It has been demonstrated convincingly that the feedback control of these changes takes place mostly locally within the retina. The nature of the control mechanisms can be probed further by using restrictive, physically defined visual stimuli, and applying transmitter agonists and antagonists to the retina.

This presentation will summarize the evidence for and against participation of the amacrine cell transmitters dopamine, acetylcholine, and glucagon, in ocular growth-control in chicks. Evidence for dopaminergic, muscarinic, and VIPergic mechanisms in primates also will be considered. Problems of delivery, availability, specificity and duration of action, and applicability of chick data to developing drug therapies for human myopia, will be discussed critically. Is there reason to suppose that a drug therapy for human myopia, directed at the retina, is worth serious consideration or effort?

PHARMACOLOGICAL INTERVENTION FOR MYOPIA CONTROL – THE SCOPE OF POSSIBILITIES AND THEIR LIMITATIONS

C Wildsoet
University of California - Berkeley

The underlying cause of both myopia and associated retinal complications is excessive ocular growth, which thus is a logical target for pharmacological intervention. The first attempts to control human myopia with drugs date back to the middle of the 19th century and involve atropine, an antimuscarinic drug. However, troubling side-effects and inconsistencies in its apparent efficacy saw a subsequent decline in the use of atropine until recently when rising myopia prevalence figures renewed interest in anti-myopia drugs generally and atropine specifically. More recent animal-based research has provided new insights into the ocular actions of atropine. This animal-based research has also opened up new possibilities for drug intervention for myopia control. This presentation will review the effects of anti-myopia drugs that have been applied clinically, to include other antimuscarinic drugs and beta-blockers, and also will consider new as yet untested treatment options identified through animal-based research. The clinical issues encountered with the pharmacological treatment of myopia in humans will also be considered in general and more drug-specific terms.

ATROPINE CONTROLLING MYOPIC PROGRESSION IN SCHOOLCHILDREN

F. Shih
National Taiwan University Hospital

Introduction: We reported lower doses of atropine would control myopia progression. Further, randomized clinical trial assessed the treatment effects of 0.5% atropine and/or multi-focal lenses in decreasing the progression rate of myopia in children.

Methods: One hundred and eighty-six children, from 6 to 13 years of age, were treated each night with different concentrations of atropine eye drops or a control treatment for up to 2 years. Another 227 schoolchildren with myopia, aged from 6 to 13 years, who were stratified based on gender, age and the initial amount of myopia were randomly assigned to three treatment groups: 0.5% atropine with multi-focal glasses, multi-focal glasses, and single vision spectacles. Each subject was followed for at least eighteen months.

Results: The mean myopic progression was 0.04 ± 0.63 diopter per year (D/Y) in the 0.5% atropine group, 0.45 ± 0.55 D/Y in the 0.25% atropine group, and 0.47 ± 0.91 D/Y in the 0.1 % atropine group. All atropine groups showed significantly less myopic progression than the control group (1.06 ± 0.61 D/Y) ($p<0.01$). Our study also showed that 61% of students in the 0.5% atropine group, 49% in the 0.25% atropine group and 42% in the 0.1 % atropine group had no myopic progression. However, 4% of children in the 0.5% atropine group, 17% in the 0.25% atropine group, and 33% in the 0. 1% atropine group still had fast myopic progression (>- 1.0 D/Y). In contrast, only 8% of the control group showed no myopic progression and 44% had fast myopic progression. The mean progression of myopia in atropine with multi-focal glasses group (0.41D) was significantly less than the multi-focal (1.19D) and single vision group (1.40D) ($p<0.0001$). But no significant difference was noted between the last two groups ($p=0.44$).

Conclusion: These results suggest that all three concentrations of atropine had significant effects on controlling myopia; however, treatment with 0.5% atropine was the most effective. The 0.5% atropine with multi-focal lenses can slow down the progression rate of myopia. However, multi-focal lenses alone showed no difference in effect compared to control.

IMMUNOLOGY AND OCULAR INFECTION

Session Chairs:
Rachel Caspi, Ph.D and
Scott Whitcup, M.D.

OCULAR IMMUNOLOGY: AN EXPANDING ROLE IN DISEASE PATHOGENESIS AND THERAPY

S. Whitcup
Allergan, Inc.

Historically, the study of ocular immunology was predominantly limited to infection and uveitis. An immune response is necessary to prevent the infected eye from wide spread tissue destruction and necrosis; however, an uncontrolled immune response can cause fibrosis and scarring that permanently impairs vision. As a result, there are unique controls on the ocular immune response such as ACAID, the anterior chamber associated immune deviation. In the absence of infection, ocular inflammatory disease can also lead to blindness. Although corticosteroids remain the mainstay of treatment for inflammatory eye disease, over the past decade a better understanding of ocular immunology has lead to improvements in therapy. These include newer immunosuppressive drugs as well as biologic agents directed against specific inflammatory cells and cytokines. More recently, inflammation has been shown to play an important role in a number of other important ophthalmic diseases including keratoconjunctivitis sicca and age-related macular degeneration. Advances in immunology and the development of medications specifically targeting components of the immune response, have also provided new therapeutic approaches for these diseases. Furthermore, the ability to deliver these drugs locally to the eye,

Disclosures:
Employee of Allergan, Inc

LIPOSOMAL DELIVERY OF SHORT-CHAIN CERAMIDE INHIBITS CXC CHEMOKINE PRODUCTION BY CORNEAL EPITHELIAL CELLS AND LIMITS DEVELOPMENT OF CORNEAL INFLAMMATION

Yan Sun, Todd Fox, Mark Kester and **Eric Pearlman**
Case Western Reserve University

The sphingolipid metabolite ceramide is generally associated with pro-apoptotic, anti-proliferative activity rather than anti-inflammatory activity. To determine the effect of exogenous C6-ceramide in corneal inflammation, human corneal epithelial cells were treated with C6 ceramide in liposome formulation (Lip-C6) prior to stimulation with inactivated S.aureus. Lip-C6, but not control liposomes inhibited production of CXCL1, CXCL5 and CXCL8. Furthermore, topical application of Lip-C6 to mouse corneas significantly inhibited S.aureus - and LPS – induced corneal inflammation as measured by neutrophil infiltration to the corneal stroma and development of corneal haze. Despite the reported activity for ceramides, Lip-C6 did not induce apoptosis of corneal epithelial cells in vitro or in vivo, nor did it inhibit corneal wound healing. Together, these findings demonstrate a novel anti-inflammatory, non-toxic, therapeutic role for liposomally-delivered short-chain ceramide, in a model of ocular inflammation and resultant visual impairment

THE ROLE OF THE IL-23 / IL-17 PATHWAY IN INDUCTION AND EXPRESSION OF OCULAR AUTOIMMUNITY

Dror Luger[1], Jun Tang, Phyllis B. Silver[1], Daniel Cua[2], Zoe Chen[2], Yoichiro Iwakura[3], Edward P. Bowman[2], Chi-Chao Chan[1] and **Rachel R. Caspi[1].**
[1]Laboratory of Immunology, NEI, NIH, and [2]DNAX, Palo Alto, CA and [3]University of Tokyo, Tokyo, Japan.

Experimental autoimmune uveitis (EAU), induced in mice with the retinal antigen (Ag) IRBP represents autoimmune uveitis in humans. The innate cytokine IL-23 promotes the proinflammatory IL-17 effector T cell response (Th17) that was reported to be critical in several autoimmune disease models, rather than the previously implicated Th1. We examined the role of the IL-23 / IL-17 pathway in ocular inflammation using 2 distinct EAU models: traditional model induced with the retinal Ag IRBP in complete Freund's adjuvant (IRBP/CFA EAU), and a newly developed EAU model induced with in vitro matured, Ag pulsed dendritic cells (DC-EAU). Using the traditional model and a series of mouse strains deficient in various components of the IL-23/IL-17 pathway or monoclonal antibodies, we defined IL-23 as a necessary and nonredundant factor in EAU pathogenesis, which is needed in the induction phase and appears critical for effector cell priming. In contrast, IL-17 appears to act in the effector stage. Interestingly, while its neutralization inhibits EAU in IRBP/CFA immunized mice, it appears that under some circumstances the IL-17-producing effector can be redundant, as a Th1-polarized cell line specific to IRBP that produces IFN-γ but no IL-17 is sufficient to induce severe EAU. Furthermore, in the alternative model of DC-EAU, presence of IL-17-producing effectors is insufficient to induce EAU in a situation where IFN-γ is lacking. These data raise the possibility that the nonredundant role of IL-23 in EAU may extend beyond its role in promoting the Th17 effector response and point to IL-23 and IL-17 as new therapeutic targets for uveitis.

BLOCKADE OF EPITHELIAL MEMBRANE PROTEIN 2 (EMP2) MAY ABROGATE CHALMYDIAL INFECTIVITY

K. Shimazaki, A. Chan, J. Braun, K. Kelly, **L. Gordon**
University of California, Los Angeles

Chlamydiae are bacterial pathogens which have evolved efficient strategies to enter, replicate, and survive inside host epithelial cells, resulting in acute and chronic diseases in humans and other animals. Although several candidate molecules involved in this process have been identified, the precise mechanism of infection has not been elucidated and the host receptor complex remains unknown. New strategies for prevention of chalmydial infections require greater understanding of the host-pathogen interaction at the receptor site(s). Epithelial membrane protein-2 (EMP2) is a 4-transmembrane protein expressed in the eye, reproductive tract, and lung, sites that are major infectious targets of Chlamydia. Here we show that chlamydial infectivity is associated with the host cellular expression of EMP2 in multiple cell lines. Recombinant knockdown of EMP2 impaired infectivity, whereas these processes were markedly augmented in recombinant EMP2 over-expressing cells. An epithelial cell line which lacks EMP2, Hs578T, is relatively resistant to chlamydial infection, and recombinant expression of EMP2 in that cell line restores chlamydial infectivity to the level observed in epithelial cells with natively expressed EMP2. Importantly, blocking surface EMP2 with anti-EMP2 antibody significantly reduces Chlamydia infection efficiency in multiple cell lines. Studies are now in progress to evaluate the utility of EMP2 blockade in abrogating Chlamydial infectivity in vivo. In this study we report that EMP2 is a newly-identified candidate host protein involved in Chlamydia infection and that blockade of the EMP2-chalmydial interaction is a potential new target for therapeutic intervention.

TREATMENT OF AGE-RELATED MACULAR DEGENERATION

Session Chair:
John Penn, Ph.D.

EFFECTS OF FENRETINIDE, A RETINOIC ACID DERIVATIVE, IN VITRO AND IN VIVO: INDUCTION OF RPE APOPTOSIS AND INCREASED CHOROIDAL NEOVASCULARIZATION

D Hinton, P Sreekumar, J Zhou, C Spee, B Maurer, R Kannan.
Keck School of Medicine of the University of Southern California

Fenretinide (N-4-hydroxyphenyl retinamide; 4-HPR) is cytotoxic to tumor cells and widely used as an anticancer drug, particularly against neurological tumors. The effect of Fenretinide in the retina is largely unknown. A recent study (Radu et al. Invest Ophthalmol Vis Sci 46: 4393-401; 2005) suggested that Fenretinide reduces serum retinol levels, and decreases lipofuscin accumulation in the retinal pigmented epithelium (RPE) suggesting its potential therapeutic use in retinal degenerations including the dry form of age related macular degeneration (AMD). We studied the cellular effects of Fenretinide in early passage human RPE particularly with reference to its apoptotic/antiapoptotic characteristics and induction of angiogenic factors. In separate in vivo experiments, the effect of chronic treatment with Fenretinide on laser-induced choroidal neovascularization was investigated. A dose-dependent increase in apoptosis (TUNEL) in incubations of RPE with Fenretinide (1-10 μM) was found. Fenretinide caused a significant, dose-dependent increase in vascular endothelial growth factor (VEGF) and a significant decrease in pigment epithelium-derived growth factor (PEDF) gene expression in RPE maintained either in serum-free or 5% serum containing media. Secretion of VEGF also increased significantly as a function of Fenretinide dose. Intraperitoneal administration of Fenretinide (0.4 mg and 1 mg twice daily for 7 and 14 days) to mice after laser-induced photocoagulation increased vascular leakage and increased CNV lesion size; both of which showed a positive correlation to the dose and tissue Fenretinide (and its metabolite) levels. In addition, there were fewer RPE cells overlying CNV lesions in mice treated with Fenretinide compared to controls as evidenced by cytokeratin staining. Significantly increased staining of RPE for the small heat shock protein alphaB crystallin was observed in Fenretinide treated mice vs untreated controls. In summary, our data indicate that Fenretinide induces apoptosis in RPE which is accompanied by upregulation of VEGF and alphaB crystallin. The augmentation of CNV with Fenretinide suggests that dosing and treatment conditions have to be carefully monitored in its therapeutic applications to AMD.

EXAMINING THE ROLE OF CYCLOOXYGENASE IN RETINAL ANGIOGENESIS

J Penn, S Yanni, M Clark
Vanderbilt Eye Institute

The cyclooxygenase enzymes (COX-1 and -2) are responsible for catalyzing the production of biologically active prostanoids from membrane-derived arachadonic acid. The cancer literature provides evidence of a role for COX and its prostanoid metabolites in tumor-related angiogenesis. Included is confirmation that non-steroidal antiinflammatory drugs (NSAID) targeting COX affectively inhibit tumor growth by reducing tumor angiogenesis. At least two mechanisms have been proposed for the inhibition of angiogenesis by NSAID: 1) inhibition of COX reduces growth factor production in hypoxia-responsive cells; and 2) inhibition of COX negatively influences the response of vascular endothelial cells to angiogenic factors. Recent findings in our lab support the notion that both mechanisms play roles in retinal angiogenesis. Employing relevant models, including retinal Müller cells and vascular endothelial cells in primary culture, as well as animal models of oxygen-induced retinal angiogenesis, we will examine the validity of targeting COX-derived prostanoids to inhibit retinal angiogenesis. The prospect of employing one agent to target prostanoid-mediated events both upstream and downstream of growth factor receptor activation holds exceptional promise for therapeutic intervention.

Disclosures:
Alcon provided one of the NSAID used in the work

AAV2-MEDIATED DELIVERY OF NOVEL ANTI-VEGF MOLECULES SFLT01 AND SFLT02

Peter Pechan 1, Hillard Rubin 1, Michael Lukason 1, Jeffery Ardinger 1, Elizabeth Barry 1, Denise Woodcock 1, Qiuhong Li 2, James Peterson 2, William Hauswirth 2, Samuel Wadsworth 1, Abraham Scaria 1
1 Gene Therapy, Genzyme Corporation, Framingham, Massachusetts (USA) and 2 Opthalmology, University of Florida College of Medicine, Gainsville, Florida (USA)

Purpose: Vascular endothelial growth factor (VEGF) plays a critical role in pathological neovascularization which is a key component of ocular diseases like wet age-related macular degeneration (AMD) or proliferative diabetic retinopathy (PDR). There are several reports of preclinical and clinical studies that demonstrate that antagonizing VEGF is a potentially useful strategy for treating such disorders. One of the most potent binders of VEGF is the VEGF receptor, Flt-1. In the current study we have engineered soluble hybrid forms of Flt-1, sFLT01 and sFLT02, that represent novel high-affinity VEGF binders with binding affinities comparable to other known high affinity VEGF binders.

Methods: Starting with the full-length Flt-1 receptor, we engineered several soluble hybrid versions. The biological activity of these constructs was determined by the HUVECs proliferation and migration assays, VEGF binding assay and BIAcore analysis. Since adeno-associated virus 2 (AAV2) mediated gene delivery offers a means to achieve local, sustained delivery of proteins into the eye we have inserted these novel anti-VEGF molecules into AAV2 vectors. The oxygen-induced retinopathy (OIR) and laser-induced choroidal neovascularization (CNV) models in mice were used to determine in vivo efficacy of these novel molecules.

Results: The molecules sFLT01 and sFLT02 have VEGF binding affinities equal or greater than full-length sFlt-1 receptor. Purified sFLT01 protein binds VEGF with affinity comparable to VEGF-Trap (Regeneron) and about 10-fold higher than Avastin® (Genentech). Transgene expression in the mouse eye following AAV2 mediated delivery was observed up to 13 months of the duration of study and no gross transgene-related toxicities were observed. Data from the oxygen-induced retinopathy (OIR) and laser-induced choroidal neovascularization (CNV) mouse models indicate that the novel anti-VEGF molecules sFLT01 and sFLT02 are potent inhibitors of retinal neovascularization.

Conclusions: We have developed novel hybrid molecules sFLT01 and sFLT02 that potently inhibit VEGF in vitro and in vivo and are being currently evaluated in multiple ocular models of pathological neovascularization.

Disclosures:
Employee, Genzyme Corporation

EFFICACY AND SAFETY OF NANOTECHNOLOGY-BASED PHOTODYNAMIC THERAPY FORAGE-RELATED MACULAR DEGENERATION USING A SUPRAMOLECULAR NANOCARRIER LOADED WITH A DENDRITIC PHOTOSENSITIZER

Tamaki Y, Tasaka F, Ideta R, Yanagi Y, Nishiyama n, Jang W-D, Harada A, Zhang G-D, Aida T, Kataoka K
University of Tokyo, School of Medicine

Exudative age-related macular degeneration (AMD), a condition caused by choroidal neovascularization (CNV), is a major cause of legal blindness in western countries. Photodynamic therapy (PDT), which utilizes cytotoxic singlet oxygen produced by photoirradiation of a photosensitizer (PS), is a most promising treatment for CNV, and is effective at reducing the relative risk of visual acuity loss; however, it requires expensive treatments and is ineffective for some groups of patient. In this study, we have developed an effective PDT for AMD employing a supramolecular nanomedical device, i.e. a novel dendritic PS, dendrimer porphyrin (DP), incorporated into supramolecular nanocarrier, a polyion complex (PIC) micelle through electrostatic interaction with oppositely charged block copolymers. The characteristic dendritic structure of the PS prevents aggregation of its core sensitizer, thereby inducing a highly effective photochemical reaction. In an attempt to deliver PS to the CNV sites, free DP and a DP-incorporated PIC micelle were examined in rats with experimental CNV. When the DP-incorporated micelle was administered, the accumulation of DP in CNV lesions was observed as early as 15 minutes after the injection, and peaked at 4 hours, and was still evident 24 hours after the injection. Whereas, after the free DP injection, DP was also recruited to CNV lesions for up to 4 hours, but disappeared within 24 hours. With its highly selective accumulation on CNV lesions, this treatment resulted in a remarkably efficacious CNV occlusion with minimal unfavorable phototoxicity in rats with experimental CNV. Furthermore, using multifocal electroretinogram, and fluorescein or indocyanine green angiography, it had almost no harmful effect on normal retina and choroid after PDT in monkeys. Our results will provide a basis for an effective approach to PDT for AMD.

TOPICAL ADMINISTRATION OF A DUAL VEGFR/JAK2 KINASE INHIBITOR REDUCES LASER INDUCED CHOROID NEOVASCULARIZATION

Z. Chen, J. Chin, G. Ibanez, S. Stoughton, D. Lohse, C. Mak, J. Cao, J. Renick, L. Dellamary, J. Yu, M. Martin, J. Hood, G. Noronha, J. Doukas, R. Soll.
TargeGen, Inc

Purpose: Vascular endothelial growth factor (VEGF) has been clinically implicated in the pathogenesis of age-related macular degeneration (AMD), and both erythropoietin (EPO) and VEGF have been linked to the pathogenesis of diabetic retinopathy (DR). We therefore sought to evaluate a novel compound (TG101095) designed to inhibit both these mitogenic pathways following its topical delivery, via inhibition of VEGF receptors (VEGFR) and JAK2 (a key signaling kinase downstream of EPO).
Methods: TG101095's ability to inhibit kinase activity was determined using biochemical-based enzymatic assays. To determine pharmacokinetic distribution, C57BL/6 mice were dosed bilaterally with 1% TG101095 eye drops (10 ?l/eye) and euthanized at 2 hours or 7 hours post dosing (n= 3 per group). Retina and choroid/sclera samples were then collected for analysis of compound concentrations using mass spectrometry. For efficacy evaluations, a murine model of laser-induced choroidal neovascularization (LCNV) was established in C57BL/6 mice (8-10 week old females). Mice were assigned to either 1% TG101095 or vehicle treatment groups (n= 12), and topical dosing initiated immediately post-laser photocoagulations (10 µl/eye, twice daily for a total of 14 days). Two weeks after laser treatments, animals were perfused with fluorescein isothiocyanate-dextran (FITC-dextran, 2x106 daltons), choroidal wholemounts prepared, and CNV area quantified using image analysis software.
Results: TG101095 inhibited both VEGFR2 and JAK2 kinases with IC50 of 38 nM and 17 nM, respectively. Delivering a single topical dose of a 1% TG101095 solution to mice achieved concentrations by 2 hr well in excess of these IC50 in both the choroid/sclera and retina (33 and 1.8 ?M, respectively). In the LCNV model, bid topical dosing with 1% TG101095 reduced neovascularization at lesion sites by 41% compared with the vehicle-dosed group (P=0.026); gross and slit lamp exams indicated that 1% TG101095 was well tolerated.
Conclusion: Single topical dosing with 1% TG101095 was sufficient to rapidly reach both choroids and retinas at concentrations approximately 1.5-log greater than that required to inhibit VEGFR and JAK2 kinases. In laser-injured mice, twice daily dosing for 14 days was well tolerated and effectively reduced lesion neovascularization. TG101095 therefore shows strong potential as a novel topical treatment for proliferative diseases such as AMD and DR.

Disclosures:
Employee of TargeGen, Inc.

TOPICALLY EFFICACIOUS MULTI-TARGETED KINASE INHIBITORS FOR THE TREATMENT OF AGE-RELATED MACULAR DEGENERATION, DIABETIC MACULAR EDEMA, AND PROLIFERATIVE DIABETIC RETINOPATHY: TG100801

R Soll
TargeGen, Inc

Age-related macular degeneration (AMD), proliferative diabetic retinopathy (PDR), and diabetic macular edema (DME) are characterized by neovascularization, vascular leakage and inflammation. To date, a topically applied therapy for these chronically-driven indications remains elusive. In this presentation we describe TG100801, the first topically applied multi-targeted VEGFR/Src kinase inhibitor to advance into the clinic, and other blended kinase inhibitors as topical treatments. Agents such as TG100801 exhibit efficacy in vivo after topical application in models of angiogenesis and permeability, and have the potential to address the underlying inflammation associated with these blinding diseases.

Disclosures:
Employee of TargeGen, Inc

NEW TREATMENT OF DIABETIC RETINOPATHY

Session Chairs:
Peter Kador, Ph.D. and
Mike Niesman, Ph.D.

DELETION OF ALDOSE REDUCTASE TO UNDERSTAND THE PATHOGENESIS OF DIABETIC RETINOPATHY

Sookja K. Chung, Alvin Cheung, Amy Lo, Kwok Fai. Presented by **Stephen Chung**
Department of Anatomy, University of Hong Kong, China

Purpose: The pathogenesis of diabetic retinopathy is still poorly understood, although hyperglycemia has been indicated to be the primary pathogenic factor. Among the proposed hypothesis, such as activation of hexosamine pathways, aldose reductase (AR), endothelin-1, protein kinase C and increased advanced glycation end product formation, the involvement of AR, which is the first and rate-limiting enzyme in the polyol pathway, received the most attention. The strongest evidence was provided by the administration of AR inhibitor to type 1 diabetic rat, which prevented basement membrane thickening, pericyte loss and microaneurysms in their retinal capillaries. Here, we attempted to further elucidate the role of AR in the pathogenesis of diabetic retinopathy by using the genetic engineering of mice to manipulate the levels of AR.

Method: The AR null mutation (AR-/-) was introduced into genetically predisposed type 2 diabetic mouse model, C57BL/KsJ-db/db (db/db), which shows signs of diabetic retinopathy, such as thickening of capillary basement membrane at 22 weeks, loss of pericytes in retinas at 26 weeks, followed by endothelial cell loss at 34 weeks.

Results: The AR-deficiency led to decreased pericyte loss (alpha-SMA staining) and fewer retinal blood vessels with the immunoglobulin G leakage (IgG staining), suggesting that AR may contribute to BRB breakdown. In addition, AR deficiency prevented diabetes-induced reduction of platelet/endothelial cell adhesion molecule-1 expression and increased expression of vascular endothelial growth factor, which may have contributed to BRB breakdown. In addition, diabetes-induced retinal oxidative stress (nitrotyrosine and PAR) and apoptosis (cleaved caspase-3), glial reactivity (S100 and GFAP) and proliferation of blood vessels (counting the number of IgG-stained vessels) were less prominent in the retina of AR-/- db/db mice.

Conclusion: These findings indicate that AR is responsible for the pathogenesis of diabetic retinopathy by triggering a cascade of retinal lesions including BRB breakdown, loss of pericytes, neuro-retinal apoptosis, glial reactivation and neovascularization.

DIABETIC RETINOPATHY: RELATIONSHIP BETWEEN RAT RETINAL CAPILLARY CELLS AND ENDOPLASMIC RETICULUM (ER) STRESS LINKED UNFOLDED PROTEIN RESPONSE (UPR)

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1Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE, 2Department of Ophthalmology, College of Medicine, University of Nebraska Medical Center, Omaha, NE, 3Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Toyama, Japan. 4Molecular Biopharmacy and Genetics, Tohoku University, Sendai, Japan.

Purpose: The endoplasmic reticulum (ER) is the subcellular compartment where membrane-spanning and secreted proteins are synthesized and many post-translational modifications occur so that proper protein folding and the formation of protein complexes take place. Alterations that lead to the accumulation of unfolded or misfolded proteins in the ER lumen lead to a cellular condition referred to as "ER stress". ER stress activated UPR is a critical signaling pathway for health and disease and has been linked to increased susceptibility to diabetes. The purpose of this study was to determine the relationship between ER stress and the viability of rat retinal capillary cells under hyperglycemic and hypoglycemic conditions.

Methods: Conditionally immortalized rat retinal pericyte (TR-rPCT) and endothelial (TR-iBRB) cell lines were cultured with DMEM medium simulating various levels of hyper- and hypoglycemia. Polyol levels were determined by HPLC. Cell Viability was assessed with a colorimetric MTS assay. TUNEL Staining was conducted using a fluorescein in situ cell death detection kit. Western blot analysis of the SDS-PAGE of the cell homogenates was conducted with antibodies against three specific enzymes for UPR (GRP78/Bip, CHOP, ATF4), procaspase-12 and the general apoptotic biomarker procaspase-3.

Results: Both pericytes and endothelial cells contain aldose and aldehyde reductase. However, under hyperglycemic conditions (25-100 mM glucose) only the pericytes accumulate sorbitol and this formation is reduced by the presence of aldose reductase inhibitor. Sorbitol accumulation was associated with increased apoptosis (TUNEL staining) but not induction of ER stress in pericytes. ER stress was also not induced by hyperglycemia in endothelial cells. In contrast, hypoglycemia was associated with the induction of ER stress and UPR linked apoptosis in both pericytes and endothelial cells.

Conclusion: It has been established by the DCCT trials that initiation of tight control is associated with an initial acceleration of retinopathy and a 3-fold incidence of hypoglycemia. Hypoglycemia – linked ER stress in both retinal capillary pericytes and endothelial cells may contribute to the initial acceleration of retinopathy as tight control is initiated.

EARLY DIABETES-INDUCED BIOCHEMICAL CHANGESIN THE RETINA: COMPARISON OF RAT AND MOUSE MODELS

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Pennington Biomedical Research Center

Aims/hypothesis. Recently, various transgenic and knock-out mouse models became available for studying the pathogenesis of diabetic retinopathy. At the same time, diabetes-induced retinal changes in the wild-type mice remain poorly characterized. The present study was aimed at comparing retinal biochemical changes in rats and mice with similar (6-wk) durations of streptozotocin-induced diabetes.

Methods. The experiments were performed on Wistar rats and C57Bl6/J mice. Retinal glucose, sorbitol, fructose, lactate, pyruvate, glutamate, alpha-ketoglutarate, and ammonia were measured spectrofluorometrically by enzymatic methods. VEGF protein was assessed by ELISA, and poly(ADP-ribosyl)ation by immunohistochemistry and Western blot analysis. Free mitochondrial and cytosolic NAD+/NADH ratios were calculated from the glutamate and lactate dehydrogenase systems.

Results. Retinal glucose concentrations were similarly increased in diabetic rats and mice, vs controls. Diabetic rats manifested ~26- and 5-fold accumulation of retinal sorbitol and fructose whereas elevation of both metabolites in diabetic mice was quite modest. Correspondingly, diabetic rats had increased retinal malondialdehyde plus 4-hydroxyalkenal concentrations, reduced superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione transferase activities, slightly increased poly(ADP-ribose) immunoreactivity and poly(ADP-ribosyl)ated protein abundance, and VEGF protein overexpression whereas diabetic mice lacked those changes. SOD activity appeared 21-fold higher in murine than in rat retina (the difference increased to 54-fold under diabetic conditions) whereas other antioxidative enzyme activities were instead 3-10-fold lower. The key antioxidant defense enzyme activities, except catalase, were increased, rather than reduced, in diabetic mice. Diabetic rats had decreased free mitochondrial and cytosolic NAD+/NADH ratios, consistent with retinal hypoxia, whereas diabetic mice preserved both ratios in the normal range.

Conclusions: Mice with short-term streptozotocin-induced diabetes lack many biochemical changes that are clearly manifest in the retina of streptozotocin-diabetic rats. This should be considered when selecting animal models for studying early retinal pathology associated with diabetes.

EXPLORATION OF INTRAVITREAL INJECTION OF LCVS1001 AS A THERAPEUTIC PROCEDURE FOR EARLY DIABETIC RETINOPATHY IN STZ-INDUCED RAT

J. Zhang, J. Shen, Y. Guan, F. Ji, S. Sinclair, Y. Luo, **G. Xu**, W. Li and G.-T. Xu
Shanghai Institutes for Biological Sciences, Chinese Acad Sci., and Shanghai JiaoTong Univ. School of Medicine

Purpose: To explore LCVS1001, a glycoprotein, for its protective and therapeutic functions to retinal vascular cells, neurons and RPE, in diabetic retinopathy in STZ treated rats.

Methods: Diabetic retinopathy was induced in rats by Streptozotocin (STZ) treatment, which leads to significant damage to retinal vascular cells, neurons and RPE cells in about a week. At the onset of diabetes, or 1 week or 4 weeks after the onset of diabetes, a single injection of various dosages of LCVS1001 (5~200 ng/eye) was given intravitreally. The eyes were then examined at different time points during the following 6 weeks. The blood-retinal barrier (BRB) was monitored by Evans blue quantification. Retinal cells death was detected by TUNEL staining. The retinal thickness and cell counts in different layers of the retina were evaluated under light microscopy. Electron microscopy (EM) was used to scrutinize retinal vascular and neuronal injury. A pharmacokinetic study of LCVS1001 following intravitreal injection was conducted. Some possible signal transduction pathways for LCVS1001 action were also studied.

Results: BRB breakdown was detected 4 days after diabetes onset, peaked at 2 weeks and then reached a plateau between 2 and 4 weeks. In the LCVS1001-treated diabetic eyes, BRB permeability was maintained at the non-diabetic control level. TUNEL-positive cells significantly increased in outer nuclear layer (ONL) at 1 week and reached the peak at 4 to 6 weeks after diabetes onset. In the LCVS1001 injected eyes there was no evident TUNEL-positive cells observed in ONL until 4 weeks after onset of diabetes. The retinal thickness, specifically, that of ONL, was reduced significantly in diabetic rats, correlating with the decrease in the cell count of ONL. Treatment with LCVS1001 prevented the cell loss and maintained a normal thickness of ONL up to 4 weeks after diabetes induction. EM examination demonstrated vascular and neuronal death starting early after the onset of diabetes. LCVS1001 administration protected the photoreceptors and capillary endothelial cells from death up to 4 weeks after diabetes onset. A dose-dependent fashion of the protective effects of LCVS1001 was observed. The pharmacokinetic profile of LCVS1001 in the vitreous fits the first order kinetics with a half-life elimination period ranging 24 to 36 hours. Several signal transduction pathways were examined for the understanding of the mechanism of LCVS1001 action.

Conclusions: Apoptosis is an important constituent of vascular and neuronal cell death in the early course of diabetic retinopathy. Timely intravitreal injection of LCVS1001 prevented the retinal cell death and the BRB malfunction, and therefore, appears to be a novel therapeutic approach for early diabetic retinopathy.

GLAUCOMA
BASIC RESEARCH

Session Chairs:
Thomas Yorio, Ph.D. and
Christopher Paterson, Ph.D, D.Sc.

GLAUCOMA THERAPY AFTER PROSTAGLANDIN ANALOGS: QUO VADIS?

Carl B. Camras
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Since their commercial availability for glaucoma therapy in 1996, prostaglandin (PG) analogs have become the leading treatment for glaucoma worldwide because of their efficacy and safety. Now, 10 years later, we continue to search for a class of drugs which offers an even better safety-efficacy profile. Although future advances might lead to treatments for glaucoma independent of intraocular pressure (IOP) lowering (e.g. neuroprotection), current treatments proven to be beneficial are restricted to IOP reduction.

Some of the many classes of drugs for IOP reduction that have been investigated in recent years include (listed alphabetically): adenosine agonists or antagonists, alpha 1-adrenergic antagonists, angiotensin antagonists, aquaporin, calcium channel blockers, cannabinoids, cytoskeletal agents (actomyosin network regulators), dopaminergic agonists, fibronectin-mediated interactions, glycosaminoglycans degradation, matrix metalloproteinase inducers, melatonin agonists, natriuretic peptides and cGMP, nitric oxide and cGMP, opioid receptors agonists, proteasome stimulators, serotonergic compounds, steroid inhibition, and trabecular meshwork cell Na+-K+-2Cl- antiporter.

Since cytoskeletal agents are the drugs that recently have been most intensively investigated on the basis of the quantity of publications in the scientific literature, they will be discussed in greater detail. These agents include all of the following: cytochalasins; ethacrynic acid (phenoxyacetic acid derivative); ticrynafen (nonsulfhydryl-reactive compound similar to ethacrynic acid); marine macrolides, including latrunculins-A and -B, swinholide A, and jasplakinolide; protein kinase C activators, including phorbol myristate acetate; protein kinase inhibitors, including H-7, HA-1077, ML-7, staurosporine, chelerythrine, rho-kinase inhibitors (e.g. rho-associated coiled coil-forming kinase [ROCK] inhibitor, Y-27632, statins [lovastatin and compactin]). These drugs increase outflow facility by the following mechanisms: disruption of the microtubule and/or actin micro-filament cytoskeleton in the trabecular meshwork (TM); rupture of the inner wall of Schlemm's canal; inhibition of microtubules; alteration of cell shape, cell-cell, and/or cell-matrix interactions; sequestration of monomeric G-actin and disassembly of actin filaments; reduction of TM cell contractility and/or prevention of elongation of actin filaments; and/or distention of the TM.

It is impossible to predict which, if any, of the above classes of drugs will eventually be approved for clinical use in glaucoma therapy. The next "new" drugs are likely to be combination products or modified drugs (e.g. with improved pharmacokinetics or delivery systems) within classes already existing for therapy. In future years, drugs are likely to be found that are effective in therapy independent of an IOP effect.

LATRUNCULINS AND OUTFLOW RESISTANCE

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Aim: Latrunculins, marine macrolides isolated from the ocean sponge Negombata (Latrunculia) magnifica, are specific and potent actin-disrupting agents that sequester monomeric G-actin, leading to the disassembly of actin filaments. Latrunculins A and B (LAT-A and -B) cause reversible dose- and time-dependent destruction of actin bundles and associated proteins in human trabecular meshwork (TM) cells. To determine if the actin cytoskeleton is involved in the regulation of outflow resistance in the TM, effects of LAT-A or -B on outflow facility and intraocular pressure (IOP), and relevant TM morphological changes after LAT-B, were studied in the live monkey eye.

Methods: Outflow facility was measured by 2-level constant pressure perfusion of the anterior chamber (AC) and IOP was measured by Goldmann tonometry in living monkeys before and after intracameral infusion or topical application of different doses of LAT-A or B. The TM was studied by light and electron microscopy following AC exchange and infusion with LAT-B (0.5µM) or vehicle plus cationized and non-cationized gold solution in opposite eyes of the monkey. The eyes were fixed by infusing Ito's solution and enucleated. Anterior segments were quadrisected and embedded in Epon-Embed 812.

Results: Both LAT-A and -B dose- and time-dependently increased outflow facility by up to 2- to 4-fold, with LAT-B being 10 times more potent than LAT-A. The increased outflow facility was reversible 3 hrs after the removal of the drug from the AC. Following a single high topical dose of LAT-A (~0.2%) or -B (~0.02%), IOP was significantly decreased by up to 3-4 mmHg, with LAT-B inducing earlier IOP reduction and smaller side effects compared to LAT-A. Multiple treatments with low doses of LAT-B (e.g., 0.01%) produced greater IOP reduction and less corneal hydration than the single treatment with 0.02% LAT-B. Morphological studies indicate that the LAT-B-induced decrease in outflow resistance is most likely due to the cytoskeleton disruption-related massive "ballooning" of the juxtacanalicular region in the TM, leading to a substantial expansion of the space between the inner wall of Schlemm's canal and the trabecular collagen beams.

Conclusions: Those findings suggest that pharmacological disorganization of the actin cytoskeleton in the TM by specific actin inhibitors such as latrunculins may be a useful anti-glaucoma strategy.

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Disclosures:
Holder of patent, consultant to company (Inspire Pharmacueticals) that has licensed the patent from WARF, research (directed and unrestricted) sponsored in my lab by Inspire)

ANALYSIS OF THE PHARMACOLOGY OF PROSTANOID INDUCED INTRAOCULAR PRESSURE EFFECTS IN NON-HUMAN PRIMATES: THE IMPORTANCE OF EXAMINING NORMAL AND OCULAR HYPERTENSIVE EYES.

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Purpose: The pharmacology of prostanoid induced effects on primate intraocular pressure has been previously analyzed but only in part. The recent reports of new selective agonists has enabled a complete analysis.

Methods: Intraocular pressure (IOP) was measured by pneumatonometry in conscious monkeys trained to accept the procedure. Both ocular normotensive and laser-induced ocular hypertensive monkeys were used. Selective agonists for each prostanoid receptor were examined .

Results: Selective agonists for CRTH2 (DP2) and EP1 receptors have recently been introduced but these drugs, 13,14-dihydro, 15-keto PGD2 and ONO-D1-004 had no effect on intraocular pressure. In contrast EP2 agonists such as butaprost, its free acid, and its 5,6-cis free acid were highly efficacious ocular hypotensive agents, similar to the prototypical EP4 agonist 3,7-dithia PGE1.The selective EP3 agonist 11,15-methoxy PGE2 had only a very modest effect on IOP.

The selective IP agonist cicaprost had a moderate ocular hypotensive effect in ocular normotensive and hypertensive eyes. The most complex IOP response occurred with the DP1 agonist BW 245C and the TP agonist U-46619. A 0.1% dose of both drugs produced an ocular hypertensive spike in normal monkeys. This was not seen in “glaucomatous” monkey eyes and both drugs behaved as ocular hypotensives, albeit
BW 245C being decidedly more efficacious than U-46619.

Discussion: Perhaps the most interesting finding was the transient ocular hypertension produced by DP1 and TP receptor stimulation in ocular normotensive monkeys. This was not seen in “glaucomatous” monkeys, where the IOP was in the 30-40 mm Hg range. Although not manifest at the high IOP level, this ocular hypertensive spike may occur at IOP levels in the typical range for glaucoma patients (20-30 mm Hg).Indeed, this response to DP1 and TP receptor stimulation has been observed in clinical studies (Nakajima M. et al., Graefe’s Arch. Clin. Exp. Ophthalmol. 229: 411,1991; Flach AJ and Eliason, J. Ocular Pharmacol. 4: 13, 1998). It is, therefore, important to examine drug effects in normal and ocular hypertensive eyes. The rank order for ocular hypotension in the “glaucomatous” monkey model was EP2=EP4>FP=DP1>IP>EP3=TP>EP1=DP2 (CRTH2).

Disclosures:
Allergan employee

VE-CADHERIN, A THERAPEUTIC TARGET FOR OCULAR HYPERTENSION

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Responding to a variety of extracellular cues, cell-cell adhesions between endothelial cells are dynamic. Assembly/disassembly of adhesion complexes between endothelial cells depends upon homotypic interactions between the extracellular domains of VE-cadherin and intracellular adaptor proteins that react to changes in their extracellular environment. In the eye, the inner wall of Schlemm's canal provides the only continuous cellular barrier to unimpeded flow of aqueous humor in the conventional outflow pathway. Thus, obstructing paracellular passageways between inner wall endothelia dramatically decreases the movement of aqueous humor out of the eye. Like other endothelia, junctions between the inner wall cells are dynamic and respond to their unique environment. In vivo, and in two different experimental model systems, cell-cell adhesions between endothelial cells of the inner wall respond to changes in intraocular pressure (pressure differentials across drainage tissues) by decreasing their complexity and enabling the formation of gaps between cells for fluid flow. We hypothesize that trapping paracellular passageways in the "open" configuration over time with monoclonal antibodies, peptide mimetics or small molecules directed against VE-cadherin will increase the movement of aqueous humor and accumulated cell debris out of the eyes of people with ocular hypertension associated with glaucoma or following cataract surgery.

Disclosures:
Advanced Glaucoma Technologies licensed patent.

CABERGOLINE: EFFICACIOUS OCULAR HYPOTENSIVE AGENT WITH MULTIPLE PHARMACLOGICAL ACTIVITIES

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Purpose: To determine the in vitro and in vivo ocular and non-ocular pharmacological properties of cabergoline.

Methods: Cabergoline and other key reference compounds were tested for their ability to modify signal transduction mechanisms by measurements of cAMP production, phosphoinositide (PI) hydrolysis and intracellular Ca2+ ([Ca2+]i) mobilization assays in cultured human ocular and other cells. In addition, cabergoline's affinity for a range of receptors, ion channels, transport sites and enzymes was determined using radioligand binding assays. Acute ocular irritation in rabbits following topical ocular dosing was determined. Intraocular pressure (IOP) was measured in conscious Dutch-Belt rabbits and ocular hypertensive cynomolgus monkeys using an Alcon pneumatonometer.

Results: Cabergoline bound to native and /or human cloned serotonin-2A/B/C (5HT2A/B/C), 5HT1A, 5HT7, a2b, dopamine-2 / 3 (DA-2/3) receptor subtypes with nanomolar affinity (IC50s = 0.5 – 17 nM). Cabergoline was an agonist at the 5HT2, 5HT1A (EC50 = 140 nM) and DA-2/3 receptors but an antagonist at the 5HT7 and a2 receptors. In primary human ciliary muscle (h-CM) and trabecular meshwork (h-TM) cells, cabergoline stimulated PI turnover (EC50 = 19 ± 7 nM in TM; 76 nM in h-CM) and [Ca2+]i mobilization (EC50 = 972 ± 50 nM in TM). It also stimulated [Ca2+]i mobilization via human cloned 5HT2A (EC50 = 63 nM), 5HT2B (EC50 = 1 µM) and 5HT2C (EC50 = 570 nM) receptors. In some experiments, cabergoline stimulated cAMP production in h-CM cells when co-incubated with forskolin (EC50 = 30-100 nM). While topical ocularly administered cabergoline failed to modulate IOP in Dutch-Belt rabbits even at 500 µg, it was an efficacious IOP-lowering agent in the ocular hypertensive cynomolgus monkeys (peak reduction of 30.6 ± 3.6% with 50 µg 3 hrs post-dose; 30.4 ± 4.5% with 500 µg 7 hr post-dose). Cabergoline caused minimal ocular irritation.

Conclusions: Cabergoline exhibited a variety of pharmacological activities in cultured cells and in radioligand binding assays. Its most prominent agonist activity involved activation of 5HT2, 5HT1A and DA-2/3 receptors, while it behaved as an antagonist at the 5HT7 and a2-adrenoceptors. Since 5HT1A agonists, and 5HT7 and a2 antagonists are not known to exhibit ocular hypotensive activities in the conscious ocular hypertensive monkeys, the 5HT2 and dopaminergic agonist activities of cabergoline probably mediated the IOP reduction observed with this compound in this species. These data suggest that cabergoline produces its efficacious IOP-lowering activity by multiple mechanisms involving [Ca2+]i mobilization and by elevating levels of cAMP in the target cells and tissues via its activation of 5HT2 and DA-2/3 receptors.

Disclosures:
Alcon Research, Ltd

NEUROPROTECTION

Session Chairs:
Yeni Yücel, M.D., Ph.D. and
Jonathan Crowston, Ph.D.

NANOTECHNOLOGY APPROACHES FOR NEUROPROTECTION AND REGENERATION OF THE CNS

Gabriel A. Silva
University of California, San Diego

Nanotechnologies are materials and devices with a functional organization engineered at the nanometre scale. The application of nanotechnology in cell biology and physiology enables targeted interactions at a fundamental molecular level. In neuroscience, and in particular applications to the CNS, this entails specific interactions with neurons and glial cells at a molecular level. Ongoing current research by several groups include technologies designed to interact with neural cells, advanced molecular imaging technologies, materials and hybrid molecules for neural regeneration, neuroprotection, and the targeted delivery of drugs and small molecules across the blood–brain barrier. In particular, this talk will introduce emerging approaches for the neuroprotection and regeneration of the CNS.

ACTIVATION OF A NOVEL NG2 GLIAL CELL EXPRESSING NMDA GLUTAMATE RECEPTOR IN THE OPTIC TRACT

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BACKGROUND: NG2 cells are a new class of glial cells recently identified within the central nervous system. These novel cells can support axons and synapses of neurons, and may express neurotransmitter receptors. NG2 cells have not been described in the optic tract or lateral geniculate nucleus (LGN) and it is unknown whether glutamate NMDA receptors exist on NG2 these cells or whether they are activated in glaucoma.

OBJECTIVES: 1. To determine whether NG2 cells exist in the optic tract and primate lateral geniculate nucleus and whether they express NMDA glutamate receptors 2. To determine whether NG2 cells are activated in experimental primate glaucoma.

METHODS: Eight monkeys with right eye unilateral experimental glaucoma with chronic elevated intraocular pressure and three normal control monkeys were studied. LGN layers were studied for evidence of NG2 immunolabeling. NG2 Chondroitin sulfate proteoglycan-positive immunoreactivity was detected using Super Sensitive Non-Biotin HRP Detection kit (BioGenex., CA, USA). Double-labelling for NG2 and NMDA receptor subunit 1 was performed with immunofluorecence using tyramide signal amplification kit (Molecular Probes, CA, USA).

RESULTS: In the normal LGN, NG2-immunoreactives star-shaped cells with branching processes were detected within and between all 6 LGN layers. NG2 cells expressed the NMDAR1 receptor subunit. In the glaucoma LGN, NG2 cells were activated with increased intensity of NG2-immureactivity and expression of the NMDAR1 receptor subunit. Similar findings were also noted in the optic tract.

CONCLUSIONS: The NG2 cells represent a novel cell type in the primate optic tract and LGN. Their activation in glaucoma suggests a role for NG2 cells in neural degeneration. The expression of the NMDA glutamate receptor on NG2 cells implicates them in both normal and abnormal stimulation of the glutamatergic system in the central visual system. NG2 cells may be a new potential target for neuroprotective treatment strategies in glaucoma.

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RETINAL TAU PATHOLOGY IN HUMAN GLAUCOMA AND POSSIBLE THERAPEUTIC IMPLICATIONS

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University of Toronto/St. Michael's Hospital

Background/aim: Tau protein is essential to microtubule integrity in neurons, and abnormal hyperphorylated tau protein AT8 is associated with neurodegenerative diseases such as Alzheimer's disease. Tau protein and abnormal tau AT8 were evaluated in human glaucoma to determine whether abnormal tau protein plays a role in glaucomatous neural degeneration.

Methods: Sections from 11 glaucoma eyes and 10 age-matched control eye specimens were immunostained for normal tau protein (BT2) and for hyperphosphorylated tau protein (AT8). Measurements of immunofluorescence intensity in glaucoma retinas were compared to those in control retinas. Abnormal tau AT8 and parvalbumin, a horizontal cell specific marker, were studied with double-immunofluorescence techniques to determine co-localization.

Results: Normal tau protein was decreased in glaucoma retina compared to age-matched control retina. Abnormal tau AT8 was evident within the posterior retina, predominantly in the inner nuclear layer in glaucoma and this was not observed in controls. Quantitative immunofluorescence techniques demonstrated significantly increased abnormal tau AT8 in glaucoma compared to controls. Abnormal tau AT8 was specifically localized in horizontal cells of the retina.

Conclusions: Abnormal tau AT8, a pathologic hallmark of neurodegenerative disorders known as the tauopathies, is present in human glaucoma. Horizontal cells in the retina are implicated in the neurodegenerative process in glaucoma.

IN-VIVO IMAGING OF RETINAL GANGLION CELLS IN THE MOUSE- OPPORTUNITIES AND THREATS

Jonathan G. Crowston
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In-vivo fluorescence imaging of the mouse retina has the potential to failitate evaluation of neuroprotective ttreatments with the longitudinal asssessment of retinal ganglion cells in mouse glaucoma models. This may be acccomplished with transgenic mice whose RGCs constitutively express fluorescent proteins or the use of exogenous fluorescent biomarkers that can identify dead or dying RGCs. The opportunities and threats to in-vivo imaging will be discussed.

INHIBITION OF PROTEIN DEACTEYLATION ALTERS MMP SECRETION FROM ASTROCYTES

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Purpose: Previous studies have provided evidence that the secretion and activation of matrix metalloproteinases (MMPs) from astrocytes contribute to retinal damage induced by inflammation, and ischemic injury. The purpose of these studies was to investigate the role of acetylation - deacetylation on matrix metalloproteinase expression and secretion in primary cultures of human astrocytes.

Methods: Primary human astrocyte cell cultures were established from optic nerve head explants and expression patterns of histone deacetylases (HDACs), and the effects of Class I and II HDAC inhibitor, TSA, on matrix metalloproteinase expression induced by the inflammatory cytokine, TNFa.

Results: The principle members expressed in these cells include HDAC1, 2, 3, 6, 9 and 11. TSA can block the induction and secretion of MMP-1 and MMP-3 induced by TNFa. These responses were dose-related with IC50s of 19 and 21 nM. The secretion of MMP required the activation of PI3-kinase/Akt signaling pathway, and initial results indicated the inhibition of HDACs disrupts these signaling events.

Conclusions: Protein deacetylation appears to play a central role in the regulation of matrix metalloproteinase expression and secretion in optic nerve head astrocytes. The use of HDAC inhibitors may provide a novel approach for the prevention and treatment of optic neuropathies.

EARLY ONSET OF OXIDATIVE STRESS IN PRESSURE INDUCED RETINAL GANGLION CELL DAMAGE IN GLAUCOMA MODELS

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University of California, San Diego

PURPOSE: To investigate the role of oxidative stress in pressure-induced retinal ganglion cell (RGC) damage, we determined the changes in the levels of two canonical markers of oxidative stress, 4-hydroxy-2-nonenal (HNE) adduct formation and heme oxygenase-1 (HO-1) expression, in response to elevated hydrostatic pressure in RGC-5 cell cultures and elevation of intraocular pressure (IOP) in mice.

METHODS: Cultured RGC-5 cells were subjected to 30, 60, or 100 mmHg elevated hydrostatic pressure for 2 hours. Mouse anterior chambers were cannulated and IOP was elevated to 30, 60, or 100 mmHg for 1 hour. After these treatments, the cells or eye tissues were either fixed or quickly harvested and frozen. Using immunocytochemistry and Western blotting, the distribution and amount of HNE-protein adducts and HO-1 expression were determined.

RESULTS: There is a dose dependent increase in level of HNE-protein adducts and HO-1 expression in pressure-treated RGC-5 cell cultures ($P<0.05$). HNE, at a concentration as low as 5 uM, leads to neurotoxicity in RGC-5 cell cultures. In recovery experiments, HNE-protein adducts in the RGC-5 cells continued to increase for up to 10 hours, whereas HO-1 expression immediately decreased. The level of HNE adducts and HO-1 expression increased in the mouse retina and optic nerve following acute IOP elevation in a strict dose-dependent manner ($P<0.05$). Antioxidant treatments can reduce the oxidative stress level.

CONCLUSIONS: This study demonstrates that oxidative stress is an early event in hydrostatic pressure/IOP-induced neuronal damage. These findings support the view that oxidative damage contributes early to glaucomatous optic neuropathy and application of antioxidants could be a potential therapeutic or preventive intervention for glaucoma.

GLAUCOMA
CLINICAL RESEARCH

Session Chairs:
Thom Zimmerman, M.D., Ph.D. and
Felipe Medeiros, M.D., Ph

CLINICAL IMPLICATIONS OF IOP FLUCTUATIONS

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Purpose: To evaluate the importance of intraocular pressure (IOP) fluctuations as a risk factor for glaucoma development and progression

Methods: Literature review and presentation of a clinical study on IOP fluctuations as a risk factor for development of glaucoma in ocular hypertensive subjects.

Results: Several studies have investigated the role of diurnal and long-term IOP fluctuations as risk factors for glaucoma development and progression. As part of the Advanced Glaucoma Intervention Study (AGIS) study, Nouri-Mahdavi et al. showed that 1mmHg increase in IOP fluctuation increased the odds of visual field progression by 30%. The Early Manifest Glaucoma Treatment Study (EMGT), on the other hand, did not find long-term IOP fluctuations to be a risk factor for glaucoma progression. In the EMGT, when mean follow-up IOP and IOP fluctuation were considered in the same time-dependent model, mean IOP was a significant risk factor for progression, whereas IOP fluctuation was not. In a recent study, we did not find long-term IOP fluctuations to be a significant risk factor for glaucoma development in a group of 105 ocular hypertensive subjects followed for an average of 84.7 ± 46.2 months. Mean (±SD) IOP fluctuation was 2.76 ± 1.16 mmHg in converters and 2.63 ± 0.86 mmHg in non converters (P = 0.548). IOP fluctuation was not a risk factor for conversion to glaucoma both in univariate (Hazard ratio [HR] = 1.19, 95% CI: 0.76 – 1.88; P = 0.461) as well as in multivariate analysis (adjusted HR = 1.42; 95% CI: 0.90 – 2.24; P = 0.130). Mean IOP during follow-up was a significant risk factor for progression both in univariate (HR = 1.12; 95% CI: 1.01 – 1.25; P = 0.041) as well as in multivariate analysis (adjusted HR = 1.16; 95% CI: 1.03 – 1.31; P = 0.013).

Conclusion: There are conflicting results in the literature with regards to the role of IOP fluctuations as a risk factor for glaucoma development and progression. Long-term IOP fluctuations do not appear to be significantly associated with risk of developing glaucoma in untreated ocular hypertensive subjects.

EFFECTS OF 24-HOUR CHANGE IN INTRAOCULAR PRESSURE ON OCULAR PERFUSION PRESSURE AND INTRAOCULAR BLOOD FLOW IN HEALTHY YOUNG ADULTS

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Purpose: There is a consistent 24-hour change pattern of intraocular pressure (IOP) in healthy young adults. The present study investigates the influences of this change pattern of 24-hour IOP on ocular perfusion pressure and intraocular blood flow.

Methods: Fifteen healthy young volunteers (age 20-25 years) were housed in a sleep laboratory for 1 day with 16-hour diurnal/wake period and 8-hour nocturnal/sleep period. Every two hours, IOP was measured using a non-contact tonometer (Ocular Response Analyzer; Reichert Ophthalmic) and systemic blood pressure was measured after 5 minutes in the sitting position. Mean blood pressure and ocular perfusion pressure were calculated. The corresponding blood flow parameters in the optic nerve head and in the macula area (flow, volume, and velocity) were measured using a scanning laser Doppler flowmeter (Heidelberg Retina Flowmeter; Heidelberg Engineering) in the sitting position.

Results: There were consistent 24-hour change patterns in IOP, mean blood pressure, and ocular perfusion pressure. However, blood flow parameters in the optic nerve head and in the macula area showed no consistent 24-hour change pattern. Nocturnal mean IOP was higher than diurnal mean IOP. Nocturnal mean blood pressure was lower than diurnal mean blood pressure. Calculated ocular perfusion pressure was lower during the nocturnal period than during the diurnal period. There was no significant difference in blood flow, volume, and velocity in the optic nerve head and macula area between the diurnal period and the nocturnal period.

Conclusions: The nocturnal elevation in IOP and the nocturnal decrease in mean blood pressure led to a decrease in ocular perfusion pressure in healthy young adults. However, there was no corresponding difference in blood flow in the optic nerve head and in the macula area. This result suggests that autoregulation can maintain the intraocular blood flow at a constant level in healthy young adults.

PRELIMINARY EVIDENCE THAT BETA2-ADRENERGIC RECEPTOR HAPLOTYPES CONTRIBUTE TO AQUEOUS HUMOR FLOW VARIATIONS IN NORMAL HUMANS

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WK Kellog Eye Center

Purpose: To test the hypothesis that beta2-adrenergic receptor (AR) haplotypes contribute to variation in aqueous humor flow in healthy subjects.

Methods: This study was designed as a prospective inpatient fluorophotometry study. Flow was measured in each eye hourly between 8 am and noon and every two hours between midnight and 6 am. Morning and nighttime flows were analyzed for differences between eyes, differences between these two times, individual concordance of flow at these two times, and association between Beta2-AR haplotypes and flow. Genotyping and haplotype reconstruction was performed on DNA using sequencing, allele specific polymerase chain reaction, and restriction enzyme digestion.

Results: In 28 subjects, the flow was similar between eyes within a subject. Using one eye per subject, the average flow was 3.12 + 1.09 microl/min in the morning, which decreased significantly to 1.59 + 0.58 microl/min at nighttime. During each time period, the flows were normally distributed. Concordance was 68% for an individual's morning and nighttime flows, which was also observed graphically by a scatter plot. Three common haplotypes (2, 4, and 6 using the nomenclature of Drysdale, CM et al., PNAS 97(19):10483, 2000) were genotyped. We observed a trend towards association of haplotype 4 with high flow and haplotype 2 for low flow in the morning.

Conclusions: In addition to affirming that aqueous humor flow is similar between eyes and that flow variation shows a normal distribution, we provide new evidence that individuals show concordance of flow in the morning and nighttime. We show preliminary observations that beta2-AR haplotypes is one of the genes that contribute to variation in aqueous humor flow. Thus, aqueous humor flow is amenable to study as a quantitative trait, which is a factor that contributes to the important glaucoma clinical risk factor of IOP variation.

CIRCADIAN VARIATION IN AQUEOUS OUTFLOW FACILITY IN YOUNG HEALTHY ADULTS

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Purpose: Recent research indicates that intraocular pressure (IOP) increases during the nocturnal period. However, the reason for this increase is unclear, since aqueous humor flow decreases at night. This study investigated whether or not changes in outflow facility at night could account for the higher nocturnal IOP.

Methods: Seventeen young healthy subjects (age 22-39 years, average 28 years) were recruited from employees and students at the Mayo Clinic (Rochester, MN) and the local area. Subjects maintained a sleep log and wore a wrist actigraph for one week prior to the study to ensure a regular sleep schedule. Subjects were housed at the Mayo Clinic General Clinical Research Center over an 18-hour period covering the mid-diurnal and mid-nocturnal periods (approximately 1 PM to 7 AM). IOP, aqueous humor flow rate, and outflow facility were measured using pneumatonometry, anterior chamber fluorophotometry, and Schiotz tonography respectively, in each eye during the mid-diurnal (2-4 PM) and mid-nocturnal (2-4 AM) periods. Nocturnal IOP, aqueous humor flow rate, and outflow facility were compared to the same variables during the diurnal period by using generalized estimating equation models to control for inter-eye correlations.

Results: In the sitting position, nocturnal IOP (13.6 ± 0.4 mmHg; mean ± SEM; n=34) was not significantly different from diurnal IOP (14.2 ± 0.3 mmHg, p=0.33). In the supine position, nocturnal IOP (18.2 ± 0.4 mmHg) was slightly less than diurnal IOP (19.6 ± 0.3 mmHg, $p<0.01$). Aqueous flow rate during the nocturnal period (1.08 ± 0.12 uL/min) was significantly decreased from flow rate during the diurnal period (2.42 ± 0.11 uL/min, $p<0.001$). Outflow facility was decreased slightly in the nocturnal period (0.25 ± 0.02 uL/min/mmHg) compared with the diurnal period (0.29 ± 0.02 uL/min/mmHg) (P=0.04). This change in facility was not sufficient to compensate for the decrease in aqueous flow rate at night. Based on the Goldmann equation, outflow facility would need to decrease by about 50% to produce the measured IOP if other parameters remained constant.

Conclusions: Outflow facility measured by tonography does not decrease enough during the nocturnal period to compensate for the decreased aqueous humor flow rate. The complete mechanism of nocturnal IOP remains to be elucidated.

THE IMPORTANCE OF IOP FLUCTUATION IN GLAUCOMA AND OF THE WDT PEAKS IN GLAUCOMA PROGRESION

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University of São Paulo

The importance of peaks and diurnal variation of IOP as risk factors to glaucomatous progression has been well established

The large fluctuations in diurnal IOP were the most important risk factor associated with visual field loss in glaucoma patients.

A recent report of the Advanced Glaucoma Intervention Study (AGIS) suggested that the mean IOP should be kept in the low teens and IOP peaks bellow 18mmHg to prevent further visual field deterioration in patients with moderate to advanced glaucomatous damage.

The best way to access this is by performing a diurnal tension curve or monitoring the patient with home-tonometry. However, problems of economics and labor make the determination of the 24h IOP course difficult, either by DTC or home tonometry. Performing various IOP measurements during the day at office hours is an alternative option. It may not provide full information about IOP fluctuation such as during the nocturnal sleep period or it may not detect peaks that occurs in different times in different days.

The Water Drinking test is another option. Although the water-drinking test has been shown to be a poor diagnostic tool in glaucoma, the emphasis on the value of this test has been changed. There is a significantly correlation between the IOP peaks in the DTC and WDT in both groups and Brubaker proposed that this test could be used as an indirect measurement of outflow facility to compare the intraocular pressure responses of glaucoma eyes to different drugs. However, the ability of the eye to recover from a transient rise of intraocular pressure secondary to water ingestion depends on the pressure sensitivity of aqueous humor outflow, the so called outflow facility. Low facility of outflow seems to account, at least in part, for the instability and larger circadian rhythm of the IOP in glaucoma patients. The WDT may be used as a surrogate marker for outflow facility reserve. It may be an indicator of the quality of the treatment and the likelihood of progression (personal communication George A. Cioffi, M.D.) . It may also be used to evaluate the risk o glaucoma progression.

A treatment that improves the facility of outflow can be expected to show less IOP variation secondary to a water challenge, and also a less IOP variation during the day.

POSTER PRESENTATIONS

Friday
February 9, 2007

CYCLODEXTRINS IN OCULAR DRUG DELIVERY

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University of Iceland

Cyclodextrins are a family of cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity. Cyclodextrin molecules are relatively large with a number of hydrogen donors and acceptors and, thus, in general they do not permeate lipophilic membranes. In the pharmaceutical industry cyclodextrins have mainly been used as complexing agents to increase aqueous solubility of poorly soluble drugs, and to increase their bioavailability and stability. Studies in both humans and animals have shown that cyclodextrins can be used to improve drug delivery from almost any type of drug formulation. However, addition of cyclodextrins to existing formulations, without further optimization, will seldom result in acceptable outcome. Through cyclodextrin complexation it is possible to formulate lipophilic water-insoluble drugs as aqueous eye drop solutions. The ocular barrier to topical drug delivery into the eye consists of the aqueous tear film and lipophilic epithelium, and most drugs permeate this barrier via passive diffusion. Cyclodextrins enhance permeation of lipophilic drugs through the aqueous tear film to the epithelial surface increasing drug availability immediate to the membrane surface. However, since hydrophilic cyclodextrins and cyclodextrin complexes do not readily permeate lipophilic membranes excess cyclodextrins will hamper drug penetration into the eye. Cyclodextrins frequently reduce drug delivery of hydrophilic drugs.

POSTER F2

EFFECT OF PERMEABILITY AND PIGMENT BINDING ON OCULAR PHARMACOKINETICS FOLLOWING TOPICAL DOSING

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Pfizer, Inc.

Purpose: The purpose of this study was to investigate the utility of in vitro corneal permeability and pigment binding assays in predicting ocular exposure. For ophthalmology projects, ocular tissue pharmacokinetic (PK) studies are important to establish the presence of the drug at the site of action and in characterizing the safety and efficacy of the compound. Both corneal permeability and pigment binding are important compound attributes that can impact ocular drug disposition following topical administration.

Methods: Existing topically administered drugs as well as discovery project compounds were tested in permeability and pigment binding in vitro assays. Permeability studies were performed using fresh excised rabbit corneas in a commercially available ussing chamber apparatus. A static pigment binding assay was used to characterize the binding of compounds to ocular melanin. Several sources of pigment including bovine, synthetic and sepia were studied in this assay. Several of the known topically administered drugs and discovery project compounds were also studied in pigmented and albino rabbit in vivo PK following topical administration. In order to characterize the distribution of the molecules, several eye tissues (cornea, aqueous humor, iris/ciliary body) were quantitated for drug levels by LC/MS/MS. For some molecules, a cassette dosing approach was utilized to decrease the variability associated with animal dosing and ocular tissue collection.

Results: Good correlation was observed between ex vivo corneal permeability and in vivo ocular exposure with a few exceptions that will be further discussed. Compounds with known permeability (beta-blockers) proved to be useful permeability markers in the rabbit ex vivo permeability model. The extent of compound binding in the pigment binding assay seems to correlate reasonably well with in vivo concentrations in ocular tissues containing melanin (iris-ciliary body). Highly pigment bound compounds appear to demonstrate the greatest difference in ocular exposure between pigmented and albino rabbits.

Conclusions: Corneal permeability and pigment binding are two important compound attributes that directly impact compound exposure in the eye. In vitro assessment of permeability and pigment binding could be useful in the design and selection of molecules with desired ocular ADME properties. Ocular exposure data for compounds that demonstrate a high degree of binding to melanin need to be interpreted with caution.

Disclosures:
Currently employed by Pfizer

NEW DIABETIC MOUSE MODELS TO TEST DRUGS FOR THE TREATMENT OF DIABETIC RETINOPATHY.

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Purpose: Transgenic and knock-out mouse models are being widely used to elucidate biochemical mechanisms involved in the development of diabetic retinopathy. Studies suggest, however, that there are significant biochemical differences between mice and rats, with diabetic mice possessing significantly lower accumulations of polyol, products of oxidative stress, and VEGF. Moreover, in rodent retinal capillaries selective pericyte destruction is difficult to identify because pericyte and endothelial capillary cells morphology is similar. Since animal studies indicate that aldose reductase activity is linked to pericyte destruction and the pathogenesis of diabetic retinopathy and polyol accumulation is linked to oxidative stress and increased VEGF levels, the purpose of this study was to develop a mouse model that expresses aldose reductase in all vascular cells containing smooth muscle actin (pericytes) along with green fluorescent proteins in these cells to aid in the identification of retinal capillary pericytes.

Methods: Colonies of transgenic animals were established from transgenic C57BL mice expressing either human aldose reductase (hAR) or green fluorescent protein (GFP) in vascular cells containing smooth muscle actin (SMAA) which had been previously prepared by Dr. Jen-Yu Tsai while at the Laboratory of Ocular Therapeutics of the National Eye Institute. These were then cross-bred to develop a mouse colony expressing both hAR and GFP in all vascular cells possessing SMAA. The presence of hAR and GFP was confirmed by genotyping each mouse through DNA isolation from tail snips and polymerase chain reaction, PCR.

Results: Staring with only 25% of offspring in each of these three colonies expressing the desired modification (GFP, hAR, or both), continuous interbreeding has resulted in an increase of expression in each group to levels >95% for the mice containing either GFP or hAR and 90% in mice expressing both GFP and hAR.

Conclusion: Following induction of diabetes in these mice, this new animal model should be useful for the study of diabetic retinopathy and the evaluation of drugs for the treatment of diabetic retinopathy.

POSTER F4

EFFICACY OF A TOPICALLY DELIVERED JAK2/VEGF-R KINASE INHIBITOR IN A MOUSE MODEL OF OXYGEN-INDUCED RETINOPATHY

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TargeGen, Inc.

Purpose: Although vascular endothelial cell growth factor (VEGF) is generally viewed as the main growth factor responsible for ocular angiogenesis, and a monoclonal antibody directed against VEGF (Lucentis) has been approved for the treatment of wet AMD, other mediators are also likely to play contributory roles. For example, erythropoietin (EPO) has recently been implicated in the angiogenic process associated with diabetic retinopathy (DR). We therefore asked whether a small molecule inhibitor of both VEGF and EPO signaling pathways could reduce retinal neovascularization in a relevant animal model.

Methods: Enzymatic assays were used to screen a novel chemical series for kinase inhibitory activity against VEGF receptors (VEGFR) and Janus kinase 2 (JAK2), a signaling element downstream of EPO. A murine oxygen-induced retinopathy (OIR) model was then used to assess any in vivo influence on retinal neovascularization. C57BL/6 mice were exposed to hyperoxia (75% oxygen) for 5 days starting on postnatal day 7 (P7), then returned to room air (21% oxygen) on P12. Test agents were delivered by topical administration for 5 days starting at P12, after which retinal flat mounts were stained with a lectin that binds murine endothelium (BSL I) and neovascular area quantified using image analysis software. Alternatively, retinas were processed for real-time RT-PCR in order to follow VEGF and EPO mRNA levels.

Results: TG101034 was selected from a novel chemical series based on its ability to inhibit both VEGFR and JAK2 (IC50= 22 and 8 nM, respectively). In the OIR model, this compound reduced retinal neovascularization by 29% ($p<0.05$, n= 11-15) relative to the vehicle control group, when delivered topically to pup eyes as a 0.1% formulation twice a day for 5 days (bidx5). RT-PCR confirmed roles for both VEGF and EPO in this model: while hyperoxia led to a 2-fold decrease in VEGF message, this then increased by 4-fold shortly upon return to normoxia before gradually falling. EPO message did not change upon hyperoxia but did spike 3-fold upon return to normoxia.

Conclusions: Oxygen cycling of mouse pups upregulates at least two important angiogenic factors, VEGF and EPO, and the topical administration of a dual JAK2/VEGFR inhibitor reduces the subsequent retinal neovascularization that occurs in this model. With further development such kinase inhibitors may represent potential treatments for DR.

Disclosures:
The presenter is an employee of TargeGen, Inc

AGE-DEPENDENT EFFECTS ON ERG IN CATDM1 (ARMD) MICE

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Allergan, Inc.

Purpose: Rakoczy, 2002, originally described a transgenic mouse (mcd/mcd) line that expresses a mutant form of cathepsin D, a lysosomal enzyme that processes photoreceptor outer segments in the retinal pigment epithelial (RPE) cells. Outer segments accumulate in RPE cells leading to hypo- and hyper-pigmentation in the fundus, accumulation of drusen-like bodies similar to those observed in age-related macular degeneration (AMD), and photoreceptor dysfunction; animals at 11 and 12 months showed a decrease in ERG amplitude. This study evaluated the time-course of ERG deficits.

Method: CatDm1 mice (2 months – 26 months old, n = 222) were dilated with tropicamide and phenylephrine and anesthetized with ketamine/xylazine, IP. The Espion® system using a mini-ganzfeld, Colorburst™ stimulator from Diagnosys, LLC was used for ERG stimulation and recording. Dark-adapted (30 minutes) mice were subjected to 3 single flash stimuli at 0.001, 0.01, 1.0 cd.s/m2, and 20 Hz flicker at 1.0 cd.s/m2. Amplitudes for B-wave were assessed at all 3 stimulus intensities, and at 1.0 cd.s/m2 for A-wave, oscillatory potentials and 20 Hz flicker. The number of animals ranged from 2 – 25 per age group.

Results: The rate of ERG decrease per month, starting at 2 months of age, was 21% up to 4 months of age, 3.5% from 5 – 11 months and 1% from 12 to 26 months. B-Wave amplitude (mean ± SD) for ages 2, 3, 4, 8, 12, 15, 20 and 24 months were 717 ± 79, 532 ± 112, 419 ± 58, 372 ± 75, 272 ± 34, 224 ± 66, 246 ± 64 and 208 ± 60, respectively.

Conclusion: The rate of ERG decline in CatDm1 mice was variable: rapid up to 4 months, followed by a slower decline to 11 months and stability up to 26 months.

Disclosures:
Allergan, Inc. Employee

POSTER F6

PLGF-1 AND EPITHELIAL HEMATO-RETINAL BARRIER BREAKDOWN: POTENTIAL IMPLICATION IN THE PATHOGENESIS OF DIABETIC RETINOPATHY

Miyamoto N, de Kozak Y, Jeanny JC, Annelise Glotin, Frederic Mascarelli, BenEzra , **Behar-Cohen F**
INSERM U598

Aims/hypothesis: Disruption of the retinal pigment epithelial (RPE) barrier contributes to sub-retinal fluid and retinal oedema as observed in diabetic retinopathy. High placental growth factor (PlGF) vitreous levels were found in diabetic patients. The purpose of this work is to elucidate the influence of PlGF-1 on human RPE (ARPE-19) barrier in vitro and on normal rat eyes in vivo.

Methods: ARPE-19 permeability was measured using transepithelial resistance (TER) and inulin flux under PlGF-1, VEGF-E, and VEGF 165 stimulation. The effect of hypoxic conditions or insulin was evaluated on TER and on PlGF-1 and VEGF receptors using RT-PCR. The involvement of MEK/ERK signaling pathways under PlGF-1 stimulation was evaluated using western blot analysis and specific inhibitors. The effect of PlGF-1 on the external hemato-retinal barrier was evaluated after its intravitreous injection in the rat eye, using semi-thin analysis and ZO-1 immunolocalisation on flat-mounted RPE.

Results: In vitro, PlGF-1 induces a reversible TER decrease and enhances tritiated inulin flux. These effects are specifically abolished by an antisense oligonucleotide directed at VEGFR-1. Exposure of ARPE-19 cells to hypoxic conditions or to insulin induces an up-regulation of PlGF-1 expression along with an increased trans-cellular permeability. The PlGF-1-induced RPE cell permeability involves the MEK/ERK signaling pathway. Injection of PlGF-1 in the rat eye vitreous induces an opening of the RPE tight junctions with subsequent sub retinal fluid accumulation, retinal oedema and cytoplasm translocation of junction proteins.

Conclusion: Our results indicate that PlGF-1 may be a potential regulation target for the control of diabetic retinal and macular oedema.

PLASMID ELECTROTRANSFER OF EYE CILIARY MUSCLE: PRINCIPLES AND THERAPEUTIC EFFICACY USING TNF-ALPHA SOLUBLE RECEPTOR IN UVEITIS

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INSERM U598

Purpose: Our purpose was to develop a non damaging electrically mediated plasmid delivery technique (electrotransfer) targeted to the ciliary muscle, which can be used as a reservoir tissue for the long-lasting expression and secretion of therapeutic proteins. We then assess the potentiality of this technology in an experimental uveitis model.

Methods: We designed electrodes and validate electrical conditions suitable for electrotransfer of the ciliary muscle. Localization of the transgene expression was evaluted thanks to GFP reporter gene expression, and kinetic of the expression in the ciliary muscle was evaluated by in vitro measurement of luminescence in ciliary muscle electrotransfered with a luciferase encoding plasmid. Clinical, histological, and TUNEL analysis were performed to ensure the safety of the procedure. EIU was induced in Lewis rats by a single footpad injection of 150 µg Salmonella Typhimurium lipopolysaccharide (LPS). Rats were treated by electrotransfer in the ciliary muscle of a plasmid encoding a chimeric TNF-alpha soluble receptor linked to the Fc fragment of IgG1 (TNFR-Is/IgG1). A single treatment was administred 6 days before the induction of the disease. Twenty-for hours after the disease induction, clinical and histological analyses were performed. Local and systemic TNF-alpha and TNFR-Is rates were also evaluated at this time point.

Results: High and long-lasting reporter gene expression was observed, which was restricted to the ciliary muscle. TNFR-Is electrotransfer led to elevated protein secretion in aqueous humor and to drastic inhibition of clinical and histological inflammation scores in rats with endotoxin-induced uveitis. No TNFR-Is was detected in the serum, demonstrating the local delivery of proteins using this method. Plasmid electrotransfer to the ciliary muscle, as performed in this study, did not induce any ocular pathology or structural damage.

Conclusion: Local and sustained therapeutic protein production through ciliary muscle electrotransfer is a promising alternative to repeated intraocular protein administration for a large number of inflammatory, degenerative, or angiogenic diseases.

POSTER F8

COMPARISON OF DIFFERENT METHODS FOR ASSESSING LASER-INDUCED CHOROIDAL NEOVASCULARIZATION (CNV) IN RATS

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Purpose: Laser-induced CNV in rats is a fast, reproducible and highly useful animal model for evaluation of compounds that inhibit angiogenesis and/or injury-induced inflammation. However, use of a single method for CNV assessment is often not adequate and reliable for predicting response to CNV-targeted therapies. In this study we sought to compare four different methods for assessment of laser-induced CNV in order to determine their suitability for use with anti-angiogenic compound screening.

Methods: The experimental CNV was induced in Brown Norway rats by rupturing Bruch's membrane of the right eyes with focal Diode (810 nm) laser photocoagulation. 3, 7, 14, 21 and 28 days after the laser injury the CNV lesion was analyzed and compared by fluorescein angiography (FA), FITC-dextran perfusion, FITC-isolection B4 staining and histological examination. A novel anti-VEGFR tyrosine kinase inhibitor AG-013958 administered intravitreally (ivt) was evaluated in this model by both fluorescein angiography and FITC-isolection B4 staining. The extent of fluorescein leakage on late-phase angiograms was scored 0-3 according to standard four grades.

Results: Positive FITC- isolection B4 staining of proliferating ECs was observed at the earliest on day 3 when the other three methods failed to detect the CNV. Vascular leakage was detectable by both FA and FITC-dextran labeling by day 7 post laser injury whereas the CNV lesions were then prominent by FITC-isolectin B4 staining and histological examination of cross sections. The retinal neovascularization peaked on day 21 and persisted through day 28. The ivt treatment of the experimental CNV with the VEGF/PDGF TK inhibitor AG-013958 inhibited the vascular leakage and neovascularization compared to vehicle controls. The leakage score graded by fluorescein angiography was significantly lower in the treated group than in the control group, which was confirmed by FITC-isolectin B4 staining.

Conclusions: Combination of FA with one other method that assesses volume of neovascularization such as FITC-dextran labeling or FITC-isolectin B4 staining might provide a pivotal approach for the screening of anti-angiogenic or anti-vascular leakage compounds. The inhibition of experimental CNV and vascular leakage seen with a VEGF/PDGF TK inhibitor strengthens the evidence for a critical role of the VEGFR/PDGFR system in the development of CNV, indicating that a selective inhibitor of the tyrosine kinases might be beneficial for the treatment of intraocular angiogenic diseases including age-related macular degeneration.

OPTICAL COHERENCE TOMOGRAPHY (OCT) AND SCANNING FLUOROPHOTOMETRY IN A RABBIT MODEL OF RETINAL ISCHEMIA-REPERFUSION INJURY

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Pharmacology, Preclinical Development, R&D, Bausch & Lomb

Purpose: To determine the utility of optical coherence tomography (OCT) and scanning fluorophotometry in evaluating retinal changes in a rabbit model of retinal ischemia-reperfusion injury.

Methods: A previously described model of retinal ischemia-reperfusion injury in rabbits was used. Retinal ischemia was induced using a hydrostatic column connected to the anterior chamber to generate total retinal and choroidal occlusion at a pressure of 140 mm Hg for one hour. OCT and scanning fluorophotometry after intravenous injection of fluorescein were performed at baseline and 1 and 7 days after the insult. Whole retinal thickness was obtained from OCT and fluorescein contents in the vitreous and the anterior chamber were obtained from scanning fluorophotometry.

Results: There was a statistically significant increase in retinal thickness at 1 day after the insult and a significant decrease (compared to baseline and day 1) at day 7. Even though there was a trend of increase in both vitreal and anterior chamber fluorescein contents at 1 day after the insult using the data from scanning fluorophotometry, the variations were too large to establish any statistical significance (n=8).

Conclusions: OCT can be used as a non-invasive tool to evaluate structural changes in the rabbit retina after ischemia-reperfusion injury.

POSTER F10

PHARMACOKINETICS OF DEXAMETHASONE FOLLOWING INTRAVITREAL IMPLANTATION OF 700 UG DEXAMETHASONE POSTERIOR SEGMENT DRUG DELIVERY SYSTEM (DEX PS DDS APPLICATOR SYSTEM) IN VITRECTOMIZED AND NONVITRECTOMIZED EYES

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APharmacokinetic & Drug Metab, BOphthalmology Clinical Research, cOphthalmology Clinical Research, 1Allergan Inc, Irvine, CA; 2Department of Ophthalmology, School of Medicine, University of California, Irvine, CA.

Purpose: Corticosteroid has been shown to clear faster in the vitrectomized compared to nonvitrectomized rabbit eyes following intravitreal administration (Retina. 2005; 25(5):556-60). The purpose of this study is to compare the ocular pharmacokinetics between vitrectomized and nonvitrectomized eyes following intravitreal administration of a biodegradable drug delivery system designed to release 0.7 mg dexamethasone (DEX PS DDS, Allergan, Inc.).

Methods: Sixty eyes of 30 Dutch-Belted rabbits (n=5/timepoint) were divided in 2 groups: 30 right eyes underwent standard pars plana vitrectomy using 25 gauge needle (vitrectomized group), and 30 left eyes were not operated on (nonvitrectomized group). All eyes received intravitreal implantation by a single dose injection of 0.7 mg DEX PS DDS. Retina, vitreous humor (VH), iris-ciliary body (ICB) and aqueous humor (AH) dex concentrations were monitored up to 30 days. Dex concentrations were quantified in rabbit and monkey samples using validated liquid chromatography/ mass spectroscopy.

Results: The rank order of dex concentration in vitrectomized and nonvitrectomized eyes was retina>VH>ICB>AH. The peak dex concentration on day 21 for retina, VH, ICB and AH samples collected from nonvitrectomized eyes was 2010-5590 ng/g, 1370 ± 1330 ng/mL, 452 ± 230 ng/g and 36.6 ± 28.3 ng/mL, respectively. The peak dex concentration on day 21 for retina, VH, ICB and AH samples collected from vitrectomized eyes was 2020-8190 ng/g, 731 ± 484 ng/mL, 228 ± 128 ng/g and 11.0 ± 6.01 ng/mL, respectively. The AUC0-30 days for the retina, VH, ICB and AH samples collected from the nonvitrectomized eyes was 35900-104000, 22400 ± 6540, 5420 ± 885, and 426 ± 105 ng•day/mL or ng•day/g, respectively. The AUC0-30 days for the retina, VH, ICB and AH samples collected from the vitrectomized eyes was 25800-105000, 14900 ± 3264, 4210 ± 880, and 185 ± 37.4 ng•day/mL or ng•day/g, respectively.

Conclusions: Unlike the faster clearance determined for intravitreal injection of corticosteroid in nonvitrectomized eyes, the pharmacokinetics of dex between vitrectomized and nonvitrectomized eyes following intravitreal administration of DEX PS DDS was similar.

Disclosures:
Allergan, Inc.
Employee

THE TOXICITY AND PHARMACOKINETICS OF INTRAVITREAL TRIAMCINOLONE

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Introduction: Intravitreal triamcinolone acetonide (TA) is widely used to treat macular disease, despite a limited duration of action and side-effects. Triamcinolone hexacetonide (TH) has been used as an alternative to TA for intra-articular injections and has been shown to provide longer-lasting effects after intra-articular injection than TA. This study assesses the relative pharmacokinetics and toxicity of intravitreal TH as compared to TA.

Methods: Three groups of 15 Dutch-belted rabbits were injected with study drug in one eye and 0.1 ml balanced salt solution in the fellow eye. The study drug in group I was TA (Kenalog) 4mg/0.1ml, commercially-available TH (Aristospan) 2mg/0.1 ml in group II, and 2mg/0.1 ml of TH with a modified vehicle in group III. Group III drug was prepared using 2mg pure TH, in suspending vehicle that contains 89.18% saline, 10.02% water, 0.75% sodium carboxymethylcellulose and 0.04% polysorbate 80. Simultaneous bilateral dark-adapted electroretinography was performed two weeks and twelve weeks after injection in ten and six rabbits per group, respectively. Saturated a-wave amplitude (asat)and maximal scotopic b-wave amplitude (bmax,scot) in study drug-injected and control eyes were compared at both time points. Three drug-injected eyes from all three groups were enucleated and frozen following euthanasia at 2, 4, 8 and 12 weeks after injection. The vitreous samples were extracted and vortexed. The supernatant was filtered, injected into an HPLC C18 column (Agilent, 4.6 x 250 mm, 5-micron, ZORBAX SB C-18), and analyzed using Shimadzu gradient high performance liquid chromatography (Shimadzu Co. Kyoto, Japan). The half-lives of the three study drugs were derived from single-exponential fits.

Results: A significant reduction in asat and bmax,scot in group II drug-injected eyes relative to BSS-injected eyes at both 2 and 12 weeks was observed ($p < 0.01$). The other groups showed no statistically significant differences at either time point. The half-life of Group I drug was determined to be 17.7 ±1.7 days, group II drug 44 ±13 days, and group III drug 12.8 ±2.3 days.

Discussion: Commercially-available TH exhibits double the intravitreal half-life relative to TA, but is toxic to the retina in this rabbit model. Specially prepared TH substituting Polysorbate-80 and carboxymethylcellulose for the commercially available vehicle is not toxic, but also does not provide the advantage of a longer half-life relative to TA. TH is a safe alternative to TA and future studies to determine relative effects on intraocular pressure and cataract are warranted.

POSTER F12

TOPICAL DEXAMETHASONE DELIVERY TO THE POSTERIOR SEGMENT OF THE EYE

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University of Iceland

Purpose: The purpose of this study is was to investigate topical drug delivery from cyclodextrin microparticles to the posterior segment of the eye using dexamethasone as a sample drug.

Methods: Aqueous 1.5% (w/v) radiolabeled dexamethasone eye drop suspension was prepared by suspending 750 mg of dexamethasone in 50 ml of aqueous solution containing benzalkonium chloride (10 mg), EDTA (50 mg), HPMC (50 mg), NaCl (100 mg) and ?-cyclodextrin (9.0 g). After heating a fine suspension (mean diameter ± SD: 20.35 ± 10.31 μm) of cyclodextrin complexes was formed. The aqueous eye drop micro-suspension was both physically and chemically stable over a seven month period. This aqueous eye drop formulation (50 μl) was administered to six rabbits (the left eye). Blood samples were collected every 30 minutes and the rabbits sacrificed after 2 hours. Then both eyes were removed and the dexamethasone concentration determined in blood, cornea, anterior sclera, posterior slera, aqueous humor, lens, iris-ciliary body, vitreous humor, retina, optic nerve and urine, using liquid scintillation counter. The relative contribution of topical permeation versus systemic delivery was determined by comparing the dexamethasone concentrations in the left and the right eyes after different routes of drug administration.

Results: Although the amount of dissolved dexamethasone in the aqueous eye drop suspension was about 0.8 mg/ml, or only about 5% of the total amount of dexamethasone in the eye drop formulation, the dexamethasone amount in the various tissues is comparable or even higher than after administration of the same amount in a solution (i.e. 1.5% dexamethasone in aqueous randomly methylated ?-cyclodextrin solution). Furthermore, the administration of the dexamethasone/?-cyclodextrin suspension increases the relative amount of drug reaching the posterior segment of the eye via the topical route and decreases the dexamethasone concentration in blood.

Conclusion: The aqueous dexamethasone/?-cyclodextrin eye drop micro-suspension resulted in more site specific drug delivery to the posterior segment of the eye compared to topical administration of the aqueous dexamethasone eye drop solution. Thus, this type of formulation can not only enhance drug delivery into the eye after topical drug administration but also decrease systemic side effects.

BIOACTIVATION AND OCULAR DISPOSITION OF TOPICALLY APPLIED PRODRUG TG100801

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TargeGen, Inc

Purpose: To investigate the bioactivation and disposition of TG100801, the first topically applied prodrug currently in clinical evaluation for the treatment of age-related macular degeneration.

Methods: The bioactivation and disposition of the prodrug TG100801 and its active metabolite TG100572 were established in a variety of experimental models. The distribution of TG100801 and TG100572 in the eye following topical administration of TG100801 was evaluated in BALB/c mice, Dutch-Belted rabbits, beagle dogs, Yucatan mini-pigs, and cynomolgus monkeys. Ocular tissues were isolated by dermal punch and/or dissection; composite concentration-time courses to 24 h post-dose for individual ocular tissues were employed for calculation of distribution kinetics. Protein binding across various species was determined; along with human eye tissue (retina, choroid) and melanin binding. Bioactivation of TG100801 to the active TG100572 using ocular tissue homogenates and recombinant enzymes.

Results: Topical ocular administration of TG100801 resulted in back-of-the-eye exposure for TG100801 and TG100572 across all species; in general, increasing the topical dose of TG100801 resulted in increased TG100801 and TG100572 exposures in back-of-the-eye tissues (e.g., choroid, retina). The esterase mediated conversion of TG100801 to the active compound TG100572 was observed in all eye tissues across species. TG100572 exhibited high binding to melanin (94.3%). TG100572 displayed extensive binding to plasma proteins, ranging from 98.1% to greater than 99.5% across species.

Conclusions: Topical instillation of the prodrug TG100801 provides back-of-the-eye exposure to the active TG100572 in all species with limited systemic absorption. Taken together, these results suggest the potential to achieve efficacious drug levels in the target tissues with a low potential for side effects associated with systemic drug accumulation.

Disclosures:
TargeGen, Inc. Employee

POSTER F14

SAFETY ASSESSMENT OF A VEGF RECEPTOR TYROSINE KINASE INHIBITOR, AG-028345, IN CYNOMOLGUS MONKEYS FOLLOWING SUB-TENON OCULAR ADMINISTRATION

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AG-028345 is a vascular endothelial growth factor receptor tyrosine kinase (VEGF RTK) inhibitor for the treatment of neovascular ocular diseases such as age-related macular degeneration (AMD). The safety of AG-028345 was assessed in cynomolgus monkeys by sub-Tenon (ST) administration at doses 1-30-fold/eye above the anticipated clinical dose. A single ST dose (intended clinical route of administration) of AG-028345 produced no macroscopic compound-related findings for up to 8 weeks of treatment in monkeys. Microscopic evaluation of ocular and systemic tissues revealed the presence of foamy macrophages with few non-foamy macrophages at the episcleral ST dose site at 8 weeks post-treatment. This finding was consistent with a foreign body reaction to the presence of AG-028345 and was not associated with tissue injury or a fulminant inflammatory response. Lymphoplasmacytic cells were observed in the choroid or extraocular muscle in the high dose group (3 of 6 animals) suggesting the presence of an active immune response. In vitro incubation of peripheral blood mononuclear cells obtained from naïve monkeys with AG-028345 for 7 days induced lymphocyte proliferation (stimulation index up to 7 compared to untreated cultures). AG 028345 also increased (1-12 fold) the in vitro production of IgM and IgG by B-cells. Collectively, AG-028345 in monkeys appears to stimulate immune cell activation that did not manifest in overt clinical symptoms in the eye or systemically at the doses and duration of the study conducted. The presence of lymphoplasmacytic cells with AG-028345 treatment is likely a compound-specific effect and not related to VEGF RTK inhibition because this reaction has not been observed for other small molecule VEGF RTK inhibitors in clinical development for AMD.

Disclosures:
Authors are employed by the company

POSTER PRESENTATIONS

Saturday
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IDENTIFICATION OF SECOND GENERATION PROSTAMIDE ANTAGONISTS (AGN 211334-6)

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1Selcia Ltd., Ongar, Essex, England , Depts. of 2Biological and 3Chemical Science s, Allergan Inc., Irvine, CA, USA , 4Covance Inc, Madison, WI, USA,

Purpose: The prostamides (prostaglandin – ethanolamides) and prostaglandin (PG) glyceryl esters are biosynthesized from the respective endocannabinoids anandamide and 2 – arachidonyl glycerol. Early studies have suggested that the pharmacological profiles of prostamide $F_{2\alpha}$ and PGE_2 glyceryl ester are unique and unrelated to prostanoid receptors. This has recently been supported by the identification of a selective prostamide antagonist AGN 204396. Here we report the activity of second generation prostamide antagonists AGN 211334,35,and 36 .

Methods: Effects on human recombinant PG receptors were studied using a FLIPR instrument: stable co-transfection of cDNAs encoding the receptors and a chimeric G protein allowed functional activity to be assessed as a Ca^{2+} signal for all receptors. The isolated feline iris was used as a key preparation where prostanoid FP receptor and prostamide activity co-exist. Potent prostamide antagonists were designed by substituting the CH_2 at position 3 with an oxygen .

Results: The prostamide antagonists AGN 211334,35,36 did not block the prostanoid FP or other PG-sensitive receptors at concentrations up to 30μM. Similarly, in the cat iris, AGN 211334-6 did not block the effects of $PGF_{2\alpha}$. The effects of prostamide $F_{2\alpha}$ in the cat iris were antagonized by AGN 211334,35,and 36, AGN 211334 being approximately 10 fold more potent than the protypical antagonist AGN 204396. $I.C._{50}$ values were AGN 211334=236nM, AGN 211335=356nM, AGN 211336=303nM, AGN 204396=2635nM

Conclusions: The identification of second generation prostamide antagonists provides further support for the prostamide receptor hypothesis. AGN 211334,35, and 36 may also provide agents sufficiently potent for studies in living animals .

Disclosures:
Selcia Ltd employee

POSTER S2

INTRAVITREAL INJECTION OF AD5.CMV.HTGF?2226/228 ELEVATES INTRAOCULAR PRESSURE AND DECREASES AQUEOUS OUTFLOW FACILITY IN THE BALB/C MOUSE

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Purpose: Intravitreal (IVT) injection in Balb/c mice of adenoviral vector encoding intrinsically active human TGFß2 (Ad5.CMV.hTGFß2226/228) yields a sustained elevation of intraocular pressure (IOP). By measuring outflow facility (C) in the mouse, we sought to establish whether a decrease in C is responsible for the IOP elevation mediated by Ad5.CMV.hTGFß2226/228.

Methods: C was measured in anesthetized male Balb/c mice (age 4-5 mo, weight 25-32 g) using a constant flow infusion method, whereby BSS-Plus® was infused (0.1 µL/min to 0.5 µL/min in 0.1 µL/min increments) through the cornea into the anterior chamber via a 30G needle and IOP determined by a WPI BLPR-2 pressure transducer. For verification of the technique, a positive control was set up whereby mice were treated with topical ocular Xalatan® (0.005%) (Pharmacia) 2 drops OU (at 25 h, 19 h, and 1 h prior to C determination). To test the effect of over-expression of TGFß2 in the AC on IOP and C, one eye of 6 mice was injected intravitreally through the equatorial sclera with a suspension of Ad5.CMV.hTGFß2226/228 (3×107 pfu/µL) in a 2 µL bolus using a 10 µL glass microsyringe fitted with a 30 G needle. On day 4 following injection and subsequent days thereafter, IOP was determined OU in each animal (TonoLab® Rebound Tonometer). On days 5 through 7, when IOP was judged to have reached the peak value in injected eyes, mice were anesthetized and C was determined.

Results: Baseline C (0.017 ± 0.001 µL/min/mmHg, mean ± SEM, n = 8 eyes) was determined as the inverse of the regression slope of the IOP-Flow Rate linear relationship (R2 = 0.965). Further, the Y-intercept (5.58 ± 0.94 mmHg) of the IOP-Flow Rate line was found to be in good agreement with expected mouse IOP while under deep anesthesia. An aqueous humor formation rate of 0.095 µL/min was estimated as C ? baseline IOP. In Xalatan®-treated eyes, C increased to 0.026 ± 0.001 µL/min/mmHg (n = 6 eyes), and was significantly greater than C measured in untreated eyes ($P < 0.001$, unpaired t-test). Peak IOP in Ad5.CMV.hTGFß2226/228 injected eyes was 24.3 ± 2.2 mmHg (n = 6 eyes), compared with uninjected contralateral eyes which exhibited corresponding IOP of 11.4 ± 0.5 mmHg (n = 6 eyes, $P < 0.001$, paired t-test). Ad5.CMV.hTGFß2226/228 injected eyes exhibited a C value of 0.007 ± 0.001 µL/min/mmHg (n = 6 eyes), compared with uninjected contralateral eyes which exhibited a C value of 0.014 ± 0.002 µL/min/mmHg (n = 6 eyes, $P = 0.005$, paired t-test).

Conclusions: In our hands determination of C in the Balb/c mouse is relatively straightforward and yields outflow facility and resting IOP values that are in good agreement with previously reported data.1,2 Topical treatment with Xalatan® increases C by ~53%. IVT injection of Ad5.CMV.hTGFß2226/228 increases IOP by ~113% and decreases C by ~50%.

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INTRAOCULAR PRESSURE-LOWERING EFFECT OF PGE2 AND PROSTANOID EP RECEPTOR AGONISTS IN MOUSE EYES

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Purpose: Prostaglandin F2alpha analogues are widely used to lower intraocular pressure (IOP), mainly through prostanoid FP receptor. However, other prostanoid receptors such as EP or DP may be also related to lower IOP. In this study, the IOP-lowering effect of PGE2 and specific agonists for EP1, 2, 3, and 4 subtypes of PGE2 receptor were investigated using mouse eyes.

Methods: PGE2 and EP1, 2, 3, and 4 specific agonists (ONO-DI-004, AE1-259, AE-248, and AE1-329) were prepared. C57BL/6J mice were acclimatized under the 12-hour light-dark cycle (6:00 on 18:00 off) for at least 2 weeks before experiments. Three micro litters of each agonist solutions were topically applied once into one of two eyes in a blind manner at 18:00. The fellow eye treated with each vehicle (5% DMSO) was served as control. IOP was measured by a microneedle method. The dose-response and time-course of IOP-lowering effect of each drug was calculated as the difference in IOP and reduction in IOP from the IOP of the non-treated fellow eye.

Results: 0.1% PGE2 decreased IOP by over 20% in 2 and 3 hour after administration. PGE2 reduced IOP in a dose-dependent manner. 0.1% EP1 and EP3 agonists had no significant IOP-lowering effect, whereas EP2 and EP4 agonists significantly reduced IOP by 21.1±6.4% and 7.5±5.5%, respectively.($p<0.01$:EP2, $p<0.05$:EP4) EP2 deficient mice were used to confirm the presence of receptor-mediated IOP-lowering effect by EP2 and EP4 agonists (n=9). EP2 agonist could not reduce IOP in EP2 deficient mice.

Conclusions: PGE2, EP2 and EP4 agonists have a potential to reduce IOP in mouse eyes, probably through their own receptors. Prostanoid EP2 and EP4 receptors may become therapeutic targets for IOP reduction.

POSTER S4

MONITORING INTRAOCULAR PRESSURE IN RODENTS USING TELEMETRY

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Purpose: Daily variation of intraocular pressure (IOP) is an important consideration in the diagnosis and treatment of glaucoma. Telemetric techniques have been successfully used to monitor 24-hour IOP patterns in conscious, freely moving median-size laboratory animals. In the present study, we applied telemetry to study 24-hour change patterns of IOP in the Sprague-Dawley rats and in the C57BL/6J and CBA/CaJ mouse strains.

Methods: Rats and mice were entrained to a daily 12-hour light and 12-hour dark cycle for at least two weeks. Miniature telemetric pressure transmitters (Data Science International, PA-C20) were implanted subcutaneously on the back of the light-dark entrained rats and mice. Tip of the pressure sensing catheter was inserted into the vitreous chamber. Telemetric data were continuously collected from conscious, freely moving rats and mice for up to 14 days. The system software was programmed to collect data at 120 Hz for 2 minutes in 5-minute intervals and the mean IOP levels in the intervals were recorded. Change patterns of IOP were also determined under an acute constant dark condition for 24 hours. Rats and C57BL/6J mice received similar surgical procedure with the pressure sensing catheter left outside the globe served as the control groups.

Results: The Sprague-Dawley rats showed a higher IOP during the nocturnal period than during the diurnal period under the standard light-dark condition (N=11). Average IOP was 23.4 ± 3.1 mmHg (mean ± SEM) during the nocturnal period and 18.1 ± 2.8 mmHg during the diurnal period. The IOP difference was 5.3 ± 1.2 mmHg. Similar 24-hour IOP pattern appeared under the constant dark condition (N=6). The C57BL/6J mouse strain also showed a higher nocturnal IOP than the diurnal IOP under both the standard light-dark condition (N=20) and the constant dark condition (N=10). The difference in IOP between the diurnal and the nocturnal periods in the CBA/CaJ mouse strain (N=21) was significantly less than in the C57BL/6J mouse strain. The control groups of 5 rats and 5 mice showed no consistent 24-hour IOP variation.

Conclusions: Circadian variations of IOP occurred in conscious, freely moving rats and mice. The nocturnal IOP was significantly higher than the diurnal IOP. The 24-hour IOP pattern persisted under the constant dark condition. The extents of diurnal-to-nocturnal IOP elevation may vary in different mouse strains.

A COMPARISON OF CIRCADIAN IOP IN RABBITS AND OCULAR HYPERTENSIVE (OHT) MONKEYS

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Purpose: Circadian IOP fluctuation is emerging as a potential risk factor for glaucomatous damage and is spurring interest in the ability to assess norturnal IOP without disturbing the subject. Nocturnal IOP has been shown to be elevated in volunteers (Liu, 1999) and glaucoma patients (Mosaed, 2004) assessed by pneumatonometry, and in rabbits (McLaren, 1996) using implanted radiotelemetry devices. The IOP elevation in humans appears to be related to supine posture. There is no corresponding published information about monkeys. This study compares the circadian IOP rhythm of rabbits, and monkeys made OHT by argon laser photocoagulation of the trabecular meshwork.

Method: Six adult normotensive Dutch-belted rabbits and 6 adult laser-induced OHT Cynomologus monkeys were used for the evaluation. A radiotelemetry device (TA11PA-C40, DSI, Saint Paul, MN) was surgically implanted under the scalp of each animal following anesthetisia with isofluorane, then the catheter was tunneled under the skin and inserted into the vitreous body and sutured in place. Animals were placed in cages fitted with antenna/receivers for data collection. Data analysis was done by ART software(DSI). Pressure readings were monitored every 5 minutes for 30 seconds at a frequence of 1000 Hz. Data was tabulated as a 1 hour running average and are presented as mean ± SD. Lights ON was at 6 am and Lights OFF was at 6 pm.

Results: Twenty-four hour IOP circadian pattern for rabbits showed higher IOP during the night compared to the day. Average nocturnal IOP was 25.2 ± 1.2 mmHg with a maximum of 26.8 ± 4.2 mmHg. Average diurnal IOP was 22.4 ± 2.1 mmHg with a minimum of 20.5 ± 5 mmHg. Primates expressed the opposite pattern: higher IOP during the day and lower IOP during the night. Diurnal IOP was 36.1 ± 0.8 mmHg with a maximum of 36.8 ± 9.8 mmHg. Nocturnal IOP was 31.1 ± 2 mm Hg with a minimum of 28.7 ± 8.1 mmHg. Rabbits and primates sleep upright.

Conclusion: Rabbits and OHT monkeys have opposite circadian IOP patterns.

Disclosures:
Allergan, Inc. Employee

POSTER S6

IN VIVO EVALUATION OF 11ß-HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN THE RABBIT EYE

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Purpose: Steroids are used in a diverse range of conditions in clinical ophthalmology and one of the most significant complications is corticosteroid-induced glaucoma, which is characterized by an increase in intraocular pressure (IOP). 11ß-hydroxysteroid dehydrogenase-1 (11ß-HSD1) is known to catalyze the interconversion of hormonally inactive cortisone to hormonally active cortisol. Carbenoxolone (CBX), an 11ß-HSD1 inhibitor, has been shown to reduce IOP in healthy volunteers. The purpose of this study was to: 1) develop an in vivo model for the assessment of cortisone to cortisol conversion in the eye and; 2) assess the PK/PD relationship of topical treatment with 11ß-HSD1 inhibitors.

Methods: Potent and selective 11ß-HSD1 inhibitors were topically administered to the rabbit eye. Two hours prior to the tissue harvest time point, cortisone was injected into the subconjunctival space in the same eye. Tissues were then evaluated for cortisone, cortisol and compound levels by LC/MS/MS. Cortisol activity was determined using a secondary mechanistic pLuc-GRE assay.

Results: The model is useful in determining the effects of topically dosed 11ß-HSD1 inhibitors on the conversion of cortisone to cortisol in the eye. Topical treatment with 11ß-HSD1 inhibitors resulted in complete inhibition of cortisone conversion to cortisol. The reduction of cortisone conversion was time- and dose-dependent as well as dependent on dosing volume (suggestive of increased spillover and washout with greater dosing volume). Contralateral effects were also noted suggesting that the inhibitor was getting to the contralateral eye by an unknown route. Changes in cortisol levels following treatment also resulted in a reduction of steroid-induced GR transcriptional activity of aqueous humor samples as assessed via pLuc-GRE assay.

Conclusions: The rabbit model is useful for the assessment of inhibition of cortisone to cortisol conversion by 11ß-HSD1 inhibitors in the eye. Topical delivery of 11ß-HSD1 inhibitors can reduce or inhibit the conversion of cortisone to cortisol. This finding coupled with the fact that CBX has been shown to reduce intraocular pressure in healthy volunteers would suggest that steroid control with 11b-HSD1 inhibitors may play a role in IOP control and could be useful in the therapeutic management of glaucoma.

NITRIC OXIDE ENHANCES THE EFFECTS OF LATANOPROST IN EXPERIMENTAL MODELS OF ELEVATED INTRAOCULAR PRESSURE

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Purpose: Nitric oxide (NO) is involved in a variety of physiological processes in the eye including regulation of regional blood flow. There is evidence that NO production is altered in a variety of pathophysiological conditions, including glaucoma. Thus, exogenous supply of NO by drug therapy is expected to produce beneficial effects such as reduction of intraocular pressure (IOP). With the aim of enhancing the IOP lowering potential of a currently used drug, we have modified the chemical structure of latanoprost (LAT) to introduce an NO-donating moiety, NCX 125.

Methods: NO-mediated activity of NCX 125 was determined in PC12 cells through measuring its ability to stimulate the formation of cGMP. NO-dependent anti-inflammatory properties were tested in RAW 264.7 macrophage cells stimulated by lipopolysaccharide (LPS). Ocular PK was examined in rabbits after topical dosing. IOP-lowering activity was determined in a rabbit model of transient ocular hypertension and in lasered hypertensive primates.

Results: NCX 125 showed NO-dependent cGMP accumulation (EC50 = 3.9 +/- 0.3 microM) in PC-12 cells and inhibited LPS-induced iNOS function in RAW 264.7 cells (IC50= 55 microM). In both assays LAT had no effects up to 100 microM. In rabbit corneal homogenate NCX 125 was rapidly hydrolyzed to LAT acid. Topical administration of NCX 125 and LAT to the rabbit eye lead to a similar time course and exposure level of LAT acid in the aqueous humor whereas LAT acid levels in the iris/ciliary body were somewhat lower for NCX 125 than for LAT (Cmax = 44 +/- 5 and 60 +/- 26 ng/g, respectively). Topical application of 0.03 % of NCX 125 blunted the intracameral saline induced IOP increase with a maximal Delta IOP of 9.7 +/- 4.7 mmHg at 60 min post-drug administration compared to vehicle. As expected, LAT was ineffective in this model. In hypertensive non-human primates, NCX 125 decreased IOP more effectively than LAT. A single topical 0.1% dose of LAT resulted in an 18% maximal reduction of IOP compared to a 30% reduction achieved with an equimolar dose of NCX 125.

Conclusions: Overall, the data obtained in validated disease models demonstrate that NCX 125, a prototype compound targeting two different mechanisms, effectively reduces elevated IOP. This finding may lead to potential new approaches for the treatment of patients at risks from glaucoma.

Disclosures:
Nicox Research Institute employee

POSTER S8

TISSUE-SELECTIVE EXRESSION OF ADENOVIRAL VECTOR IN MONKEY ANTERIOR SEGMENT

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Alcon Research, Ltd.

Purpose: Intraocular injection of adenoviral vectors (AdV) has been used to transduce cells in the anterior segment. Most of the studies were conducted in rodents. In this report, we characterized the expression of AdV in the anterior segment of the monkey after intravitreal injection.

Methods: Two adult male cynomolgus monkeys received a single unilateral intravitreal injection of an AdV vector (Ad5.CMV) encoding the green fluorescent protein (GFP). The injection volume was 20 µL with a viral titer of 4?106 pfu/?L in one animal and 4?107 pfu/?L in the other. GFP expression in the eye was evaluated by in vivo ocular examination and fluorescence photography. At Day 6 after injection, the animals were euthanized. Frozen cross-sections of the eyes were assessed by fluorescence microscopy.

Results: GFP expression in the monkeys was obvious from Days 2-6 after intravitreal injection of AdV.GFP. There was no significant difference in GFP expression between the two vector titers. At Day 3, with in vivo ocular examination, a distinct ring of intense green fluorescence was clearly discernible in the limbus of the eye. No fluorescence was detected in other regions. At Day 6 the fluorescence appeared to spread to the iris and cornea, such that under UV illumination, a more diffused green haze permeated the anterior segment. Histological examination of these eyes revealed that GFP was selectively expressed in the anterior chamber angle, especially the trabecular meshwork, as well as the anterior epithelium of the iris. In addition, a small number of cornea endothelial cells were also GFP positive. The corneal epithelium, ciliary body, lens, and retina did not express any detectable level of GFP. No fluorescence was visible in the non-injected eyes.

Conclusions: Intravitreal injection of AdV.GFP in the monkey induces GFP expression in the anterior segment, especially in the trabecular meshwork and iris epithelium. This technique demonstrates the feasibility of viral transgene expression in selective tissues of the eye and should be useful in research of ocular diseases, such as glaucoma.

Disclosures:
Alcon employee

THE REICHERT OCULAR RESPONSE ANALYZER AS A TOOL FOR DETERMINING MEDICATION REGIMENS AND PATIENT COMPLIANCE

David Luce
Reichert Inc.

Purpose: To demonstrate the clinical advantages of tracking glaucomatous patients on IOP control therapy utilizing the measurement capability, archiving and display functions of the Ocular Response Analyzer (ORA), Archived data can be displayed and reviewed using a number of user-selectable time-plots for trend analysis.

Method: Glaucomatous patient data, taken from a large ORA database, was reviewed (data courtesy Dr. Mitsugu Shimmyo). Patients with a minimum of eight office visits during a one-year period were evaluated. Data from two of these patients was selected for this presentation. Various time plots of the four ORA measurement parameters, along with measurement response signals, are presented.

Results/Discussion: The Ocular Response Analyzer (ORA) measures, archives and graphically presents a Goldmann equivalent IOP (IOPG), a corneal compensated IOP (IOPCC), corneal hysteresis (CH), corneal resistance factor (CRF). The raw corneal deformation signals are also archived and appear to contain clinically significant characteristics. The practical aspects of organizing and interpreting several measurement parameters recorded over a number of office visits is best accomplished by visual (versus numeric) presentation. Automatic recording of data reduces potential recording errors. To date, no instrumentation has been available that measures, archives and presents longitudinal information in a simple and convenient manner that gives the practitioner a clearer understanding of treatment efficacy and patient compliance
Examples of IOP, CH and waveform evolution over time demonstrate that the ORA signals are highly irregular at early stages of IOP therapy and "normalize" as sustained IOP control is achieved. The ORA's IOPCC has been demonstrated to be less dependent on corneal properties than IOP measurements by Goldmann and, as such, may be a better indicator of the actual IOP. In addition, recent studies have demonstrated that glaucoma and its progression are related to reduced CH. Therefore, its history may provide an added dimension in determining therapy effectiveness.

Disclosures:
Reichert Inc. - employee

POSTER S10

IN VITRO EFFICACY OF AN OPHTHALMIC DRUG COMBINATION AGAINST COMMON EQUINE CORNEAL PATHOGENS

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Purpose. To evaluate the in vitro efficacy of an ophthalmic drug combination against common equine corneal pathogens.

Methods. Alpha-hemolytic staphylococcus, ß-hemolytic staphylococcus, Pseudomonas aureginosa, Aspergillus and Fusarium spp. were subjected to minimum inhibitory concentration (MIC) testing, in triplicate, of the drug combination and each of its components. The combination consisted of equal volumes of Natacyn® 3.33%, Tobramycin 0.3%, Cefazolin 5.55% and neat equine blood serum. Proteinase inhibitory activity of the drug combination was compared to that of serum alone using a fluorescence microplate assay. MIC differences were compared using paired t-tests. Proteinase inhibition differences were analyzed using a 2-way ANOVA test. P-values less than 0.05 were considered statistically significant.

Results. The mean ± standard deviations of each medications' MICs were as follows, where (X, Y) represents (MIC of medication when tested alone, MIC of medication when tested as a component of the drug combination). Values are listed in µg/ml, with * denoting statistically significant differences within pairs. Cefazolin for a-hemolytic staphylococcus spp.: (1.33 ± 0.58, 1.70 ± 1.51), cefazolin for ß-hemolytic staphylococcus spp.: (1.33 ± 0.58, 0.83 ± 0.00), cefazolin for P.aureginosa: (> 512 ± 0.00, 144.40 ± 232.23); tobramycin for a-hemolytic staphylococcus spp.: (10.83 ± 18.33, 0.09 ± 0.08), tobramycin for ß-hemolytic staphylococcus spp.: (0.25 ± 0.00, 0.05 ± 0.00)*, tobramycin for P.aureginosa: (43.00 ± 73.61, 7.88 ± 12.67); natamycin for Aspergillus spp.: (128.00 ± 0.00, 16.65 ± 0.00)*, natamycin for for Fusarium spp.: (32.00 ± 0.00, 8.33 ± 0.00)*. The drug combination was as effective as serum at inhibiting proteinase activity for four hours, reducing protein destruction by 30.3% and 41.2%, respectively. After 4 hours the drug combination was a significantly less effective inhibitor than serum alone.

Conclusions. A drug combination consisting of equal volumes of natamycin 3.33%, cefazolin 5.5%, tobramycin 0.3%, and neat equine serum is as effective in vitro as its individual components against the common equine corneal microbes tested in this study. This combination is also as effective as serum alone at inhibiting proteinase activity for up to 4 hours under the conditions tested. Drug combinations have the attractive potential to minimize the time, stress and fatigue associated with topical treatment regimens for equine ulcerative keratitis. The in vitro efficacy proven by this study supports anecdotal reports of clinical efficacy with drug combinations, and justifies further clinical testing.

TO WHAT EXTENT DOES A LACK OF REFRIGERATION OF GENERIC CHLORAMPHENICOL EYE DROPS USED IN INDIA DECREASE THEIR PURITY AND WHAT ARE THE IMPLICATIONS IN UNITED STATES AND EUROPE?

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Background/aims: Thermal degradation of chloramphenicol occurs at a faster rate when stored in unrefrigerated conditions. This study measures the concentration of the principal thermal breakdown product of generic chloramphenicol being sold over the counter in chemists located in Delhi and Chennai (Madras) in India.

Methods: 48 samples of generic chloramphenicol eyedrops were collected form Delhi and Chennai (Madras) in India. Conditions of storage of chloramphenicol eyedrops were recorded at the time of purchase. Concentrations of a hydrolytic degradation product of chloramphenicol were measured using validated high pressure liquid chromatography in a UK drug manufacturing unit at Moorfields Eye Hospital, London. Results were compared to accepted UK standards.

Results: Significantly higher levels of chloramphenicol thermal breakdown product were found in collected samples. All samples purchased were being stored in unrefrigerated conditions in the chemists sampled. Shelf lives exceeded UK equivalents, varying considerably between manufacturers.

Conclusion: Inadequate refrigeration and prolonged shelf lives of chloramphenicol generics collected from Delhi and Chennai are associated with very high levels of chloramphenicol thermal breakdown product. These levels substantially exceed UK quality assurance standards undermining product reliability, contributing to the possible positive selection of resistant organisms and product toxicity effects. The principals of quality assurance breakdown described are applicable to Europe, following recent de-regulation of chloramphenicol eyedrop purchase over the counter, to patients worldwide storing medication at home and to medical care workers treating patients travelling from India.

POSTER S12

REDUCTION OF PRE-OPERATIVE CONJUNCTIVAL BACTERIAL FLORA WITH THE USE OF MUPIROCIN NASAL OINTMENT

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Purpose: Nasal carriage of gram positive organisms is a risk factor for surgical site infections. Multiple studies have shown that nasal application of Mupirocin ointment reduces the rate of nasal gram positive organism carriage, which in turn has been shown to reduce the incidence of post-operative infections in cardiothoracic, orthopedic, and other surgical patients. The purpose of this study was to determine whether the use of Mupirocin ointment for pre-operative eradication of nasal bacterial carriage was effective in reducing conjunctival bacterial flora.

Methods: In this prospective, blinded clinical trial, 42 patients (eyes) undergoing intraocular surgery were randomized to either control or study groups. Nasal cultures were obtained in all patients. Patients in the study group received pre-operative Mupirocin ointment applied to each nostril for 5 days prior to surgery. All patients received a standard 5% povidone-iodine prep before the surgical procedure and conjunctival cultures were obtained in all patients before and after the povidone-iodine prep.

Results: All 42 patient nasal swabs were positive for bacterial growth. Two of 20 eyes (10%) in the study group had positive conjunctival cultures prior to povidone-iodine prep, compared with 9 of 22 eys (41%) in the control group ($p<0.05$). Even after the povidone-iodine prep, 8 of 22 eyes (36%) in the control group demonstrated persistent positive cultures, while only 1 eye (5%) in the study group exhibited growth ($p<0.05$).

Conclusions: Prophylactic use of Mupirocin nasal ointment resulted in significant reduction of conjunctival flora, with or without pre-operative topical 5% povidone-iodine prep. The use of Mupirocin nasal ointment prior to intraocular surgery is a novel method for reducing conjunctival contamination rates, which in turn should reduce the incidence of post-operative endophthalmitis.