Vision Research Day
Friday, June 11, 2010
IBEW
Pittsburgh, PA

WELCOME!

We are proud to host Vision Research Day at the IBEW in Pittsburgh, PA, sponsored by the UPMC Eye Center and the University of Pittsburgh School of Medicine Center for Continuing Education in the Health Sciences (CCEHS).

If there is any way that we can be of assistance or if you have any questions regarding the meeting, please do not hesitate to ask.

Siobahn A. Gallagher
Academic Program Manager

Lauren Wally
Manager, Administration & External Affairs

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OVERVIEW

WHO SHOULD ATTEND

This course is designed for physicians practicing in the area of ophthalmology, as well as nurses and other health care professionals.

COURSE TOPICS AND OBJECTIVES

At the conclusion of the symposium, participants should be able to:

1. List the current research activities of the trainees within the Department of Ophthalmology at UPSOM.
2. Identify opportunities for future research within the Department of Ophthalmology at UPSOM.
3. Recognize the importance, opportunities for, and challenges related to the pursuit of a career within academic ophthalmology.
4. Understand the importance of the psycho-social issues affecting the patient who has suffered a severe eye injury, and be aware of appropriate sites for referral.

CONTINUING EDUCTION CREDIT

The University of Pittsburgh School of Medicine is accredited by the ACME to provide continuing medical education for physicians.

The University of Pittsburgh School of Medicine designates this educational activity for a maximum of 5.5 *AMA PRA Category 1 Credits™*. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Other health care professionals are awarded **5.5** continuing education units (CEU's) which are equal to **5.5** contact hours.

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SCHEDULE

Friday, June 11, 2010

7:30 AM Registration / Breakfast

8:00 AM Introduction
Dr. Schuman and Dr. Waxman

8:10 AM **Guillermo Amescua, MD**
Immune Cell Interactions within Rejecting Murine Corneal Grafts

8:25 AM **Alethea Hein, MD**
Comparative Chemical Analysis of Branded Latanoprost with Generic Latanoprost Formulations, instead of Chemical Analysis of Branded Latanoprost with Generic Latanoprost

8:40 AM **Jeremy Wingard, MD**
Antibiotic Efficacy to Protect Ocular Surface Cell Lines against Subsequent Clinical *Staphylococcus aureus* Challenge is Demonstrated using a Novel Cell-Associated Protection Assay (CAPA)

8:55 AM **Amar Joshi, MD**
Evaluating the Ability of Stem Cells to Maintain Viability in the Keratotomy Plane of LASIK

9:10 AM **Break**

9:25 AM **Leanne Labriola, DO**
The Red Eye Diagnostic Instrument (RED-I)

9: 40 AM **Kimberly Miller, MD**
Cataract Surgery Case Times at the VA Hospital

9:55 AM **Eric Wu, MD**
The Effect of Azithromycin 1% in DuraSite® On Biofilm Formation in *Staphylococcus aureus* and Coagulase Negative Staphylococcus

10:10 AM **Ellen Mitchell, MD**
Pupillary Light Reflexes in Premature Infants

10:25 AM **Divya Mutyala, MD**
A Novel Surgical Technique for Deep Anterior Lamellar Keratoplasty

10:40 AM **Break**

10:55 AM **Susruta Lecture – Victor Perez, MD**
The Clinician Scientist Approach to Understand and Treat Ocular Surface Diseases: From Immunity to Keratoprosthesis

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11:55 AM **Question and Answer Session**

12:00 PM **Lunch and Poster Session**

1:30 PM **Michael Pokabla, DO**
Evaluation of Anterior and Posterior Segment Camera's For Application in Tele-Ophthalmology Consultations

1:45 PM **Rajiv Shah, MD**
5 Year Experience of Acute Retinal Necrosis at UPMC Eye Center

2:00 PM **Katherine Zamecki, MD**
Bacterial Isolates in Dacryocystitis at UPMC for the past 3 years

2:15 PM **Nancy Buchser, MD**
Retinal Nerve Fiber Layer (RNFL) Thickness Measurement Reproducibility Comparison across Three Spectral Domain Optical Coherence Tomography (SD-OCT) Devices with Systematic Bias Correction

2:30 PM **Rodolfo Vicetti Miguel**
Aqueous Humor Selectivity Inhibits Inducible Nitric Oxide Synthase (NOS2) Activity in Intratumoral Macrophages through a Post-Translational Mechanism

2:45 PM **Danny Roh**
Corneal Endothelial Changes in DNA Repair-Deficient Mice

3:00 PM **Divya Gupta, PhD**
Role of Kruppel-Like Factor 4 in Mouse Lens Gene Expression, Development and Function

3:15 PM **Question and Answer Session**

3:30 PM **Adjournment**

Poster Session

Jongsick Kim
Normalization of Time Domain Optical Coherence Tomography (TD-OCT) Retinal Nerve Fiber Layer (RNFL) Thickness Measurements at Variable Scan Locations to a Virtual Universal Center Location using Three Dimensional (#D) Spectral Domain (SD-) OCT Data

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Michelle Gabriele

Distribution of Gold Nanorods Monitored with Spectral-Domain Optical Coherence Tomography (SD-OCT) after Intravitreal Injection

Peter Brennen, MD

Drusen Area Calculations on Fundus and Corresponding *En-face* OCT Images: A Comparison

Jared Knickelbein, PhD

Expression and Function of Natural Killer (NK) Cell Receptor in Ocular HSV-1 Infection

Allison Ungar

A Comparison of Objective and Subjective Quantitative Parameters at the Initial Visit to Predict Future Glaucomatous Visual Field (VF) Progression

Akshar Abbott

Ethyl Pyruvate's Effects on the TGF-B1-Induced Expression of a-Smooth Muscle Actin and Fibronectin in Human Tenon's Capsule and Trabecular Meshwork Cells

Doreswamy Kenchegowda, PhD

Regulation of Gastokine-1, Uroplakin-1B and Uroplakin-3B Promoter Activities by KLF4, KLF5 and Oct1

Xuan Li, PhD

CD44V6 Mediates Migration of Corneal Stromal Fibroblasts

Kristin Rarey, MD

The Optos Imaging System as an Ophthalmoscope: Fundus Assessment of Problem Eyes Where Conventional Examination Techniques Fail

Veeral Shah, MD, PhD

Longitudinal Analysis of Sequential Morphological and Visual Acuity Data in Patients with Dry Age-Related Macular Degeneration (AMD) to Identify Key Predictors for the Conversion to Wet AMD

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FACULTY

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FACULTY DISCLOSURE

Faculty for this activity have been required to disclose all relationships with any proprietary entity producing health care goods or services, with the exemption of non-profit or government organizations and non-health care related companies.

The following information was disclosed:

Dr. Victor Perez - Receives grant and research support from Alcon Laboratories. Dr. Perez is a consultant for Alcon, Inspire, and Promedior. Dr. Perez is also on the speakers' Bureau for Alcon and Inspire.

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Cellular Interactions and Architecture within Inflamed Murine Corneas

Guillermo Amescua, MD, Jared E. Knickelbein, PhD, Robert L. Hendricks, PhD.

Purpose:

CD4 T cells are integral to the immunopathology of both corneal graft rejection and herpes stromal keratitis (HSK). However, the exact cellular interactions responsible for eliciting CD4 T cell effector functions within the cornea during these disease states remain incompletely understood. The naïve murine contains a structured stratification of antigen-presenting cells with MHC class II^+ dendritic cells (DC) anchored within the epithelial basement membrane, MHC class II^{lo} $CD11b^+$ presumed macrophages in the anterior stroma, and MHC class II^- $CD11b^+$ macrophages populating the remainder of the stroma. The goal of the current study is to define and contrast the cellular interactions and architecture within corneas inflamed due to either graft rejection or HSK.

Methods:

Fluorescence confocal microscopy was used to image full-thickness corneal graft tissue from mice who received syngeneic (Balb/c to Balb/c) or allogeneic (C57B6 to Balb/c) corneal transplants, or whose corneas were scarified and infected with herpes simplex virus type 1 (HSV-1) to induce HSK. In some experiments, transgenic mice that express enhanced green fluorescent protein (EGFP) from the CD11c promoter (pCD11c) were used to visualize pCD11c-active DC.

Results:

Infiltration of CD4 T cells was observed in corneas with HSK following HSV-1 infection and in rejected corneal allografts. Infiltration of pCD11c-active DC was more dense in rejected corneal allografts than was observed in corneas with HSK. Conversely, corneas inflamed secondary to HSV-1 infection contained substantially more $CD11b^+$ presumed macrophages compared to rejected corneal allografts. Consistent with these findings, CD4 T cells appeared to interact predominantly with $CD11b^+$ macrophages in HSK corneas and with pCD11c-active DC in rejected grafts. Interestingly, MHC class II expression was confined to the epithelial and endothelial layers and was essentially absent from the edematous stroma of rejected corneal grafts.

Conclusion:

While corneal immunopathology caused by graft rejection and HSK are both mediated principally by CD4 T cells, the cellular interactions and architecture within the cornea of these distinct diseases appear to be quite different. Further understanding of the exact cellular interactions within inflamed corneas may allow development of more effective therapeutic interventions for these blinding pathologies.

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Comparative Chemical Analysis of Branded Latanoprost with Generic Latanoprost Formulations

Alethea Hein, MD, Michael Pokabla, DO, MS, Dujuan Lu, MS, Lei Hong, PhD, Xiaomi, Xu, MS, Stephen Weber, PhD, Robert Noecker, MD, MBA

Purpose:
To compare the concentrations of latanoprost and preservative benzalkonium chloride (BAK) in branded latanoprost (Xalatan, 0.005% latanoprost with 0.02% BAK) and two commercially available generic formulations (Latoprost, labeled as 0.005% latanoprost with 0.04% BAK, and 9 PM, labeled as 0.005% latanoprost with 0.02% BAK).

Design:
Analytical chemical analysis of three available latanoprost eye medication preparations.

Participants:
None

Methods:
Reference solutions of latanoprost and BAK were prepared. An optimized High Performance Liquid Chromatography (HPLC) determination was developed and used on Xalatan and two different formulations of generic latanoprost each advertised with a concentration of 0.005% latanoprost.

Main Outcome Measures:
The latanoprost and BAK concentrations in branded and generic latanoprost ophthalmic solutions.

Results:
The concentrations of latanoprost found in two generic formulations (Latoprost and 9 PM) were 97.0% ± 8.5% ($p=0.37$) and 92.2% ± 7.4% ($p=0.02$) of that in branded latanoprost (Xalatan) respectively. Concentrations of the preservative BAK were also measured as 0.23 ± 0.001, 0.25 ± 0.006 and 0.22 ± 0.02 mg/mL for Xalatan, Latoprost and 9 PM respectively.

Conclusions:
Some variability in concentrations of latanoprost and its preservative exists among available formulations. Further studies are necessary to determine the clinical significance of this variability.

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Antibiotic efficacy to protect ocular surface cell lines against subsequent clinical *Staphylococcus aureus* challenge is demonstrated using a novel Cell-Associated Protection Assay (CAPA)

Jeremy Wingard, MD; Eric Romanowski; Regis Kowalski; Francis S. Mah, MD; Jerry Gordon, MD; Robert Shanks, PhD

Purpose:
Standard MIC determination may not optimally evaluate the effectiveness of certain antibiotics in protecting the ocular surface against bacterial infection. In particular, antibiotic association with epithelial cells may play a key role. We used a previously described assay to evaluate the cell-associated efficacy of azithromycin (AZ), erythromycin (ER), tetracycline (TET), and bacitracin (BAC). Antibiotic toxicity was also evaluated.

Methods:
Chang conjunctival and human corneal limbal epithelial (HCLE) cells were grown to confluence in 96-well plates using antibiotic-free media. Cells were washed and incubated in triplicate in media containing AZ, ER, TET, and BAC (0-512 μg/ml). After 24 hours, cells were washed 2X and challenged with 6 clinical conjunctivitis/blepharitis *S. aureus* isolates ($5x10^5$ CFU) without antibiotics in the culture media. After another 24 hours, bacterial growth and epithelial cell layer survival were assessed. Bacterial viability was determined by culture turbidity (A=600 nm) and growth on blood agar plates. Epithelial cells were stained with gentian violet, with positive staining representing intact monolayers. After imaging each plate, dye was solubilized and measured at A=590 nm. Antibiotic toxicity was determined with alamar blue. Experiments were repeated at least twice per cell line.

Results:
Incubation of Chang and HCLE cells with AZ, ER, and TET at ≥64 μg/ml provided protection against challenge with AZ-susceptible *S. aureus* strains, with increasing protection at higher concentrations. This was shown in turbidity assays and confirmed by bacterial outgrowth from supernatants as well as visual observation and quantitative analysis of gentian violet staining. TET toxicity was demonstrated at 64 μg/ml, whereas no other antibiotic demonstrated consistent toxicity even at 512 μg/ml.

Conclusions:
A spectrum of protective efficacy against *S. aureus* challenge was displayed by the four antibiotics tested, with consistent results across all assays. AZ, ER, and TET were all protective, but TET also demonstrated toxicity. BAC did not demonstrate protection. *In vivo* effectiveness of antibiotic therapy for conjunctivitis and blepharitis may depend on the ability of the antibiotic to associate with epithelial cells to provide continued protection.

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Implanted Human Corneal Stromal Stem Cells Attach and Persist Under LASIK-like Flaps in Recipient Corneas

Amar Joshi, MD, Danny Roh, James Funderburgh, PhD

Introduction:

LASIK (laser in-situ keratomileusis) is a refractive surgical procedure in which a hinged corneal stromal flap is created and then replaced following laser stromal ablation. Despite its popularity, there are limitations to the safety and efficacy of the procedure such as the risk of flap dehiscence. There are currently no widely accepted methods to prevent flap dehiscence or restore biomechanical strength to flaps in uncomplicated LASIK surgery patients. Human corneal stromal stem cells (hCSSC) have recently been isolated which exhibit clonal growth, expression of the stem cell markers ABCG2 and PAX6, and display multipotent differentiation capability. These cells have a vast potential for modulating corneal wound healing in a variety of settings.

Purpose:

As part of a broader effort to uncover the regenerative potential of hCSSC on corneal healing after LASIK surgery, this study examined the persistence and attachment of hCSSC implanted under LASIK-like flaps created in organ-cultured goat corneas. Given the long term persistence of these cells after injection in murine corneas *in vivo* [11], we hypothesized that hCSSC placed under a corneal flap would remain attached to the host corneal stroma.

Methods:

Freshly enucleated goat eyes were used to create whole-organ corneal cultures. LASIK-like flaps were created in each cornea and DiO fluorescently labeled hCSSCs were implanted under the flaps. The corneas were allowed to incubate for 12, 36, 60, or 84 hours, after which, the flaps were lifted and the corneas placed into a wash solution. The solution/corneas were agitated to dislodge non-adherent cells. The corneas were removed and fixed, and the cells in the wash solution were collected and fixed. The cells in solution were counted using a hemacytometer and the corneas were qualitatively analyzed.

Results:

Mean cell counts from the washed cells substantially increased after the 12-hour timepoint and thereafter remained stable. In all of the corneal specimens, a significant number of labeled hCSSC, which were originally deposited onto the stromal bed of the corneal flap, remained within the flap interface through 84 hours in culture. There were no fluorescent cells seen outside of the interface in any of the specimens. The hCSSC appear to gather at or near the flap edges as well along the ridges in the interface made by the scalpel during flap creation. Morphologically, the cells seen in the flap interface corresponds well with the appearance of labeled hCSSC in cell culture.

Conclusion:

This study shows that hCSSCs implanted under a goat corneal LASIK-like flap persist in organ culture as long as 84 hours. However, we did not see an expected decline in labeled hCSSC in wash solution over time. Importantly we have also established a useful methodology to test the ability of hCSSCs to modulate post-LASIK wound healing in *ex vivo* living cornea organ cultures.

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The Red Eye Diagnostic Instrument (RED-I): A structured approach to assessing common non-traumatic red eye conditions in an acute care setting

Leanne Labriola, DO, Evan Waxman, MD, PhD

Introduction:

The non-traumatic red eye is a common condition that presents to emergency departments. A large majority of non-traumatic red eye conditions can be handled by emergency medicine physicians and do not need specialty consultation. However, these conditions must be differentiated from other sight threatening diseases that do require subspecialty consultation. Prior studies have shown that implementing guidelines in a clinical setting can help to improve diagnosis and management of disease, even in regards to ocular disease. This study investigates the utility of implementing an algorithm for use by emergency medicine residents in their approach to common red-eye conditions in the context of a case-based module.

Methods:

The emergency medicine residents at the University of Pittsburgh Medical Center were enrolled in a prospective randomized trial. The study was an assessment consisting of eight common non-traumatic red eye cases. Each case had a chief complaint, history, physical exam findings, and three slit lamp photographs. The experimental group was presented with the algorithm, called the Red Eye Diagnostic Instrument (RED-I), which consisted of a set of eight yes/no questions that highlight the important signs and symptoms of the exam. Each case asked for the appropriate diagnosis and disposition. A similar study was conducted concurrently at St. Barnabas Hospital in New York with the same investigators. An end survey was given to all subjects. Expert opinion was used as the gold standard for the module. Ophthalmology residents severed as the positive control.

Results:

At St. Barnabas Hospital, subjects tested with access to the 8-question algorithm obtained the correct diagnosis 15.1% more frequently (p-value 0.010) than residents who took the red eye module with only prior knowledge and no structured approach. When asked for appropriate disposition, subjects in the St. Barnabas group who were using the structured approach performed only 2.5% better (p-value 0.744). Subjects at the University of Pittsburgh with access to the 8-question algorithm did not show a statistical difference as compared to subjects without access to the algorithm in regards to diagnosis (68.3 % to 76% respectively) or disposition. Results from the end-survey showed that most residents see an average of between 5-10 red eye cases/month, but their last formal training in the non-traumatic red eye was more than 12 months prior, and most found prior training insufficient.

Conclusion:

Ophthalmological training is important for emergency medicine physicians. Current training is insufficient and ineffective. The Red Eye Diagnostic Instrument may be a useful educational tool in that can help establish the correct diagnosis of common non-traumatic red eye diseases but further validation of the module must be performed.

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The Effect of Azithromycin 1% in DuraSite® on Biofilm Formation in *Staphylococcus aureus* and Coagulase Negative Staphylococcus.

Eric Wu, MD

PURPOSE:

To analyze the effect of azithromycin 1% ophthalmic solution in DuraSite® (AzaSite®; Inspire Pharmaceuticals Inc., Alameda, CA) on biofilm formation in Staphylococcus aureus and coagulase negative Staphylococcus (CNS).

METHODS:

Four clinical strains of S. aureus and CNS, each with two azithromycin susceptible and two azithromycin resistant strains, were obtained and plated with serial dilutions of four treatment groups – azithromycin 1% ophthalmic solution in DuraSite® (AzaSite®), azithromycin 1% in 15 mM sodium citrate buffer, benzalkonium chloride (BAK) 0.0375%, and DuraSite®. After 20 hours of incubation at 37º C, total bacterial growth was quantified using a spectrophotometer detecting absorbance at 600 nm. Plates were stained with crystal violet and biofilm formation was quantified using a spectrophotometer detecting absorbance at 590 nm.

RESULTS:

Azithromycin susceptible strains demonstrated a statistically significant reduction in planktonic bacterial growth at all studied dilution strengths of AzaSite® and azithromycin 1% ($p<0.001$). There is a corresponding statistically significant reduction in biofilm formation in the same treatment groups ($p<0.05$). However, resistant strains treated with AzaSite® at concentrations between 1% and 0.125% demonstrated a statistically significant reduction in biofilm formation compared to controls ($p<0.005$) while planktonic growth did not appear to be affected. Azithromycin 1% did not affect planktonic growth in azithromycin resistant strains but did have a small but statistically significant effect on biofilm formation at concentrations between 1% and 0.125% in most tested strains. DuraSite® was noted to have no significant impact on planktonic growth at any dilution but had a statistically significant reduction in biofilm formation at concentrations of 1%, 0.5%, and 0.25% in all studied bacterial strains ($p<0.05$). BAK inhibited both planktonic growth and biofilm formation between concentrations of 0.0375% and 0.0042% ($p<0.005$) but at concentrations of 0.0023% and 0.0012% the presence of planktonic bacterial growth correlated with the presence of biofilm formation which was not significantly different from controls.

CONCLUSIONS:

As expected, when bacterial growth was inhibited by azithromycin, AzaSite®, or BAK, biofilm formation was prevented. Consistent with studies with gram-negative bacteria, azithromycin inhibited biofilm formation at sub-inhibitory doses. This is the first observation of this with gram-positive bacteria. Unexpectedly, DuraSite®, while having no effect on planktonic bacterial growth, significantly inhibited biofilm formation at concentrations between 1% and 0.25% on all strains. Our data indicates that while azithromycin 1% has a modest inhibitory effect on biofilm formation, the ophthalmic drug delivery system DuraSite® plays a more significant role in the inhibition of biofilm formation by AzaSite®.

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Pupillary Light Reflexes in Premature Infants

Ellen Mitchell, MD, Joana Osario, MD, Christin Sylvester, DO, Micheal Painter, MD, Valeria Fu, PhD, Katherine Hinch, RNC, MS,

Introduction:

In the current medical literature there is little information regarding pupillary response in premature infants. Information regarding pupillary responses in premature infants provides relevant information regarding the visual and neurological systems.

Purpose:

To add to the understanding of development of the neuro-ophthalmological system in premature infants.

Methods:

Pupillography is performed to measure the pupillary responses to light, peripheral neuromuscular integrity of the iris pupillary sphincter muscle is tested using pilocarpine eye drop (for eligible patients), visual evoked potentials is performed to study the myelinization of the optic pathway and cardiorespiratory monitoring will be done to study the heart rate variation as a measure of the autonomic nervous system development.

Conclusion:

This will be the first study determining the presence of the consensual pupillary reflex and investigating the mechanisms responsible for the presence of pupillary light reflexes in this population. It will also provide important information about visual evoked responses and autonomic nervous system development in premature infants.

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A Novel Surgical Technique for Deep Anterior Lamellar Keratoplasty

Divya Mutyala, MD, Deepinder Dhaliwal, MD

Purpose:
To describe a novel surgical technique and the clinical outcome of deep anterior lamellar keratoplasty (DALK) in a patient with previous Intacs implantation

Methods:
Interventional Case Report

Results:
One patient with previous Intacs segment implantation underwent a big-bubble assisted DALK procedure. The DALK was successfully completed without prior explantation of the Intacs segments. There were no surgical complications.

Conclusion:
DALK may be proposed as an alternative to penetrating keratoplasty for the treatment of patients with poor visual outcomes after Intacs implantation. The big-bubble assisted DALK procedure may be successfully executed with the Intacs segments in place.

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Susruta Lecture

The Clinician Scientist Approach to Understand and Treat Ocular Surface Diseases: From Immunity to Keratoprosthesis

Victor Perez, MD

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Evaluation of Portable Imaging Modalities for Use in Tele-Ophthalmology Consultations

Michael Pokabla, DO, Robert J. Noecker, MD

Introduction:

Ophthalmology coverage in many hospital and emergency room settings may not be available for a multitude of reasons including: scarcity of physician resources, remote geographic location, or weather variability. This void in coverage may result in delayed diagnosis, delayed care, or financial strains to the hospital and patient. This pilot study was to evaluate the image quality, camera portability, and ease of use of an anterior and posterior segment camera for application in ophthalmology telemedicine consultations.

Methods:

Anterior segment imaging was created using the Total Exam™ S-Video Examination Camera (GlobalMedia Group, LLC., Scottsdale, AZ, USA). Imaging of the anterior segment was transmitted and evaluated at a separate location using Easy Share Video Conference Software, version 1.9.1400 (GlobalMedia Group, LLC. Scottsdale, AZ, USA). Posterior segment imaging was acquired using The Provizion Panoptic Ophthalmoscope Anterior Segment and Retinal Camera Attachment™ (Provizion USA, WI, USA) attached to a PanOptic Ophthalmoscope (Welch Allyn Inc., Skaneateles Falls, NY, USA). Posterior segment imaging was evaluated using Logitech webcam software version 12.0.1280.0000 (Logitech, Fremont, CA, USA).

Results:

Image quality, camera portability, and ease of use of the two cameras were found to be feasible for application in the hospital or emergency room setting. Notable limitations in evaluating the posterior segment included media opacities and poor papillary dilation.

Discussion:

The images acquired with these cameras may offer medical providers the necessary support to aid in the diagnosis, treatment, or triage of patients through real time consultation with an ophthalmologist. No current standardized imaging system is available in hospitals or clinics to provide ophthalmology consultations.

Conclusion:

Further studies are needed to evaluate the validity of these two cameras in a hospital and emergency room setting to provide effective ophthalmology telemedicine consultations.

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Acute Retinal Necrosis Secondary to HSV

Rajiv Shah, MD and Andrew Eller, MD

Purpose:
To show that acute retinal necrosis (ARN) secondary to HSV may be more virulent than ARN resulting from VZV. In our experience, these cases have increased vitreous inflammation, develop more complex retinal detachments, and require more aggressive medical therapy in order to induce retinal healing.

Methods:
Case series of 6 patients presenting with ARN, diagnostically proven to be secondary to HSV with PCR testing of the aqueous humor.

Results:
All patients were initially treated with IV or oral acyclovir as well as a series of at least 3 intravitreal injections of foscarnet. After two weeks of therapy, there was no further progression of the ARN lesions, but the initial lesion failed to resolve completely. In addition, there was increasing vitritis with loss of fundus details, in spite of oral prednisone. In two of our patients, teenage males presenting with ARN and a history of neonatal herpes, lesion resolution was not seen after 1 month of IV acyclovir and later responded to 3 doses of cidofovir. For 3 of the cases, surgical intervention was necessary because the patients developed hypotony and ultrasound evidence of retinal detachment. In each of these cases, there was extensive preretinal fibrosis affecting the peripheral retina, and surgery was unsuccessful, even with a silicone oil tamponade. The later three cases received earlier and more elaborate surgical intervention, and were successful.

Conclusions:
In our experience, ARN secondary to HSV may be more virulent requiring closer observation with more aggressive medical and surgical management to obtain successful outcomes.

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Bacterial Isolates in Dacryocystitis at UPMC for the Past Three Years

Katherine Zamecki, MD, S. Tonya Stefko, MD, Jenny Y. Will, MD

Introduction

Acute and chronic dacryocystitis are very common infectious processes encountered by ophthalmologists. Knowing the bacteriology of there conditions is important in selecting appropriate antibiotic treatment. Studies have shown that the causative pathogens in acute dacryocystitis are most commonly gram positive organisms. However, gram negative organisms are isolated in a significant portion of cases and this must be considered when selecting antibiotic coverage.

Purpose

To identify bacterial isolates and antibiotic sensitivities in cases of acute and chronic dacryocystitis in adult patients.

Methods

Retrospective chart review of acute and chronic dacryocystitis cases from 2007-2010 treated at Eye and Ear or affiliated clinical sites. Charts to be reviewed were selected based on diagnosis codes for either acute or chronic dacryocystitis. Only patients who had culture and sensitivity information were then included. Bacterial isolate(s), antibiotic sensitivities, patient age, acute or chronic dacryocystitis, presence of diabetes, and presence of immunosuppression were recorded. Cultures were taken directly from the lacrimal sac at the time of surgery or from material expressed from the punctum.

Results

Twenty-six cases of acute and chronic dacryocystitis meeting the criteria for the study were identified. Age range of patients was fifteen to eight-seven. Twelve patients were female (46%) and fourteen were male (54%). There were fourteen cases of acute dacryocystitis and eleven cases of chronic dacryocystitis. There was one case of acute on chronic infection. Five patients were identified as having diabetes, while three were immunocompromised at the time of diagnosis. Of the twenty-six cases, twenty-two had positive bacterial cultures. Twenty cases were mono-microbial while two were poly-microbial. Gram positive organisms accounted for 73% of cases. Gram negative organisms accounted for the remained. There were five cases of methicillin-resistant staphylococcus aureus (MRSA), of which four were in patients with diabetes. There was no correlation found between presence of diabetes or immunosuppresion and infection with gram negative organisms.

Conclusion

Gram positive organisms remain the most common etiologic agents in acute and chronic dacryocystitis. This is consistent with previous reports in the literature. In diabetic patients, it may be important to consider infection with MRSA.

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Retinal Nerve Fiber Layer (RNFL) Thickness Measurement Reproducibility Comparison Across Three Spectral Domain Optical Coherence Tomography (SD-OCT) Devices with Systematic Bias Correction

Nancy Buchser, MD, Hiroshi Ishikawa, MD, Gadi Wollstein, MD, Rick Bilonick, MD, Larry Kagemann, PhD, Robert J. Noecker, MD, Eiyass Albeiruti, MD, Joel S. Schuman, MD, FACS.

Purpose:
To compare the RNFL measurement reproducibility across three SD-OCT devices using their native default scan protocol and analysis while correcting for systematic measurement bias.

Methods:
Forty eyes of 20 healthy volunteers underwent peripapillary RNFL thickness measurements using three SD-OCT devices: Cirrus HD-OCT optic nerve head (ONH) cube 200x200 protocol (Carl Zeiss Meditec, Dublin, CA); RTVue ONH protocol (12 radial lines and 6 concentric circles of various diameters, each centered on the ONH; Optovue, Fremont, CA); 3D OCT-1000 3D Scan 256x256 protocol (Topcon, Paramus, NJ) on the same day. All images were qualified for the manufacturer recommended image quality. Global and quadrant RNFL measurements from all three SD-OCT devices were analyzed for bias (systematic error) and imprecision (random error) using the method of maximum likelihood for a non-constant bias model.

Results:
Global mean RNFL thickness measurement showed estimated imprecision (scale bias corrected) of 2.76 μm (Cirrus), 7.76 μm (RTVue), and 4.36 μm (3D OCT-1000). In other words, RTVue was 2.8x more imprecise and 3D OCT-1000 was 1.6x more imprecise than Cirrus in global RNFL measurement. Raster scan pattern of Topcon tended to have the greatest imprecision while the same scan pattern performed by Cirrus with a shorter scanning time provided the least imprecision.

Conclusions:
RNFL thickness measurements vary between scanning sectors and among imaging devices. Faster dense 3D scanning may have an edge over other sampling methods.

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Aqueous Humor Selectivity Inhibits Inducible Nitric Oxide Synthase (NOS2) Activity in Intratumoral Macrophages Through a Post-Translational Mechanism.

Rodolfo D. Vicetti Miguel, Tom Cherpes, Kyle C. McKenna, PhD

Purpose:
Determine mechanisms that inhibit NOS2 activity in macrophages infiltrating intraocular tumors.

Methods:
Ovalbumin (OVA) specific CD8+ CTL were intravenously transferred into C57Bl/6 mice 6 or 10 days after injection of OVA-expressing tumors (E.G7-OVA) in the anterior chamber or skin respectively. Six days later intratumoral macrophages were isolated and analyzed for tumoricidal activity and nitric oxide (NO) production. Flow cytometry was used to determine expression of NOS2 and Arginase I (Arg1). Peritoneal and RAW 264.7 macrophages were stimulated in vitro with IFN-γ and TNF or LPS in the presence or absence of aqueous humor (AqH). Nitrite concentration was then determined as an indirect measure of NO production. NOS2 activity in cell lysates was measured by conversion of L-arginine to L-citrulline. RT-PCR for NOS2, GTP cyclohydrolase, CAT2B, Arg1 and Arg2 mRNAs was performed. Flow cytometry was employed to determine protein expression of NOS2, Arg1, CD40, PDL-1, MHCII, IL-12 and TNF.

Results:
Intratumoral macrophages isolated from ocular tumors but not skin tumors of CTL transferred mice were impaired in their ability to produce NO despite NOS2 protein expression. Similarly, peritoneal macrophages and RAW 264.7 cells stimulated in the presence of AqH produced significantly less NO despite NOS2 protein expression. Decreased NO production was the result of inhibited NOS2 activity which was not due to increased Arg1 activity or inhibited GTP cylohydrolase activity because mRNAs for these molecules were not affected by AqH and supplementation of cultures with L-arginine and tetrahydrobiopterin did not overcome AqH-mediated NO suppression. TGF-β2, CGRP and α-MSH alone or in combination did not recapitulate the suppressive effect of AqH on NO production by macrophages. Macrophage expression of CD40, PDL-1, MCH-II, IL-12 and TNF were increased upon stimulation and were not affected by AqH, indicating selective inhibition of NOS2 activity.

Conclusions:
Reduced NO production by ocular tumor associated macrophages was due to inhibition of NOS2 activity rather than decreased NOS2 protein expression. A similar phenomenon was observed when macrophages were stimulated in the presence of AqH suggesting that the ocular microenvironment is capable of inhibiting macrophage effector function. The effect of AqH on NOS2 activity was not mediated by TGF-β2, CGRP or α-MSH challenging current dogma.

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Corneal Endothelial Changes in DNA Repair-Deficient Mice

Danny Roh, Y. Du, PhD, A.R. Robinson, Michelle Gabriele, L.J. Niedernhofer, James L. Funderburgh, PhD

Purpose:

The accumulation of unrepaired DNA damage is known to cause degenerative changes in numerous tissues that correlate significantly to changes that occur spontaneously with aging. Our previous study (IOVS 2008;49:4837-43) demonstrated the accumulation of DNA damage in corneal endothelial (CE) cells treated in vitro with the genotoxic mitomycin-C, used in refractive surgery. This led us to study the effects of DNA damage on CE in vivo. Using mice genetically modified to express reduced levels of Ercc1-XPF (Ercc1-/?), a DNA endonuclease required for nucleotide excision repair of bulky monoadducts and the repair of interstrand crosslinks, we sought to identify long term changes in the CE resulting from the accumulation of DNA damage.

Methods:

Ercc1-/? mice and control littermates were examined at 4-5 months of age. Wild type "old" (24 months) and "young adult" (3 months) mice were also examined. Spectral-domain optical coherence tomography (Bioptigen, Inc., Durham, NC) was used for in vivo anterior segment and retinal imaging, while confocal microscopy (Confoscan 3, Nidek Tech., Padova, Italy) was used for detailed corneal imaging. Globes were fixed in PFA for histology and whole-mount staining. Endothelial cell counts were performed and 3-D reconstructions of serial confocal images created with Metamorph.

Results:

The changes in the Ercc1-/? mice CE were similar to those of the wild type "old" mice examined. Ercc1-/? mice displayed decreased CE cell density, increased CE cell pleomorphism and polymegathism, and the presence of guttae-like excrescences relative to controls. 3-D reconstructions demonstrated that the guttae-like excrescences projected into the anterior chamber originating from the CE monolayer. Significant increases in corneal thickness or opacity based on our imaging were not observed.

Conclusions:

DNA repair deficiency accelerates the degeneration of CE cells in vivo. Despite these degenerative changes, the CE in Ercc1-/? mice appears to be able to maintain the proper hydration balance of the cornea. These results support the conclusion that DNA damage may play a causal role in the degeneration of CE.

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Role of Krüppel-Like Factor 4 in Mouse Lens Gene Expression, Development and Function

Divya Gupta, PhD, Doreswamy Kenchegowda, PhD, Joram Piatigorsky and Shivalingappa K. Swamynathan, PhD

Purpose:
We have previously reported that the Klf4-conditional null (Klf4CN) lens is defective. Here, we have compared the wild type (WT) and Klf4CN lens gene expression patterns to understand the role of Klf4 in the lens gene expression, function and development.

Methods:
Developmental expression of Klf4 was studied by Q-PCR using standard curve method. Lens morphology was studied by light and electron microscopy. WT and Klf4CN lens gene expression patterns were compared by microarray, validated by RT-PCR and analyzed using BRB-ArrayTools and Ingenuity Pathway Analysis tools. Influence of KLF4 on selected promoter activities was measured by cotransfection assays. Fluorimetric method was used to measure glutathione levels in WT and Klf4CN lens.

Results:
Expression of Klf4, first detected in the embryonic day-12 (E12) mouse lens, peaked at E16, and steadily declined with age. Average diameter of the 8 week-old Klf4CN lens was 58% of the age matched WT lens. Klf4CN lens developed structural deformity and central opacity, unlike the normal WT lens. Gene expression comparison by microarray identified 226 and 276 genes up- and down-regulated by more than 2-fold, respectively, in the Klf4CN lens. Alox12 and Alox15 were up-regulated while several crystallins were down-regulated in the Klf4CN lens. Co-transfection with pCI-Klf4 stimulated the Alox12, Alox12e, Alox15 and Shsp/□B-crystallin promoter activities by 4- to 20-fold. Pathway analysis identified the lipoxygenase pathway as one of the significantly affected pathways in the Klf4CN lens. Reduced glutathione (GSH) levels were lower while oxidized glutathione (GSSG) levels were higher in the Klf4CN lens than the WT, indicating that the Klf4CN lens is oxidatively stressed.

Conclusions:
The expression of Klf4 is developmentally regulated in the mouse lens, where it contributes to different pathways including arachidonic acid metabolism, aryl hydrocarbon receptor signaling, and glutathione metabolism.

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Normalization of Time Domain Optical Coherence Tomography (TD-OCT) Retinal Nerve Fiber Layer (RNFL) Thickness Measurements at Variable Scan Locations to a Virtual Universal Center Location Using Three-Dimensional (3D) Spectral Domain (SD-) OCT Data

Jong S. Kim, Hiroshi Ishikawa, MD, Juan Xu, PHD, Gadi Wollstein, MD, Richard A. Bilonick, PhD, Larry Kagemann, PhD, Joel S. Schuman, MD, FACS

Purpose:
To develop and test a method of normalizing TD-OCT RNFL thickness measurements obtained at variable scan locations to a virtual universal center location using a retinal nerve fiber bundle distribution (RNFBD) pattern detected on the corresponding 3D SD-OCT images.

Methods:
Twelve eyes of 12 healthy subjects and 7 eyes of 7 glaucoma subjects were enrolled. A set of nine TD-OCT (Stratus OCT; Carl Zeiss Meditec, Inc., Dublin, CA (CZMI)) circumpapillary scans (one centered and 8 intentionally off-centered) were obtained for each eye. One 3D SD-OCT (Cirrus HD-OCT; CZMI) optic nerve head cube scan was also obtained at the same visit. RNFBD pattern was modeled by detecting the major RNFBD curvatures (one for each in superior and inferior hemi-field) on a SD-OCT cube data for each eye. RNFL thickness measurements from off-centered TD-OCT scans were normalized by using the modeled RNFBD pattern and the matched scan location within the corresponding SD-OCT cube data. Algorithm performance was assessed by comparing global and sectoral RNFL thickness measurement imprecisions with and without normalization.

Results:
RNFL thickness measurement imprecision was statistically significantly lower with normalization than without in all sectors except for global mean.

Conclusions:
The developed normalization method reduced the RNFL thickness measurement variability caused by variable scan locations. This method may be useful for longitudinal glaucoma progression analysis.

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Distribution of Gold Nanorods Monitored with Spectral-Domain Optical Coherence Tomography (SD-OCT) After Intravitreal Injection

Michelle L. Gabriele, Gadi Wollstein, MD, Kyle C. McKenna, PhD, Hiroshi Ishikawa, MD, Larry Kagemann, PhD, Joel S. Schuman, MD, FACS

Introduction:

Gold nanorods (GNRs) have the potential to be useful for contrast enhancement within optical coherence tomography images. However, the distribution of GNRs in the eye after intravitreal injection has not been documented.

Purpose:

The goal of this study was to monitor the location of GNRs using SD-OCT and confirm the location with transmission electron microscopy (TEM).

Methods:

GNRs were prepared using a well established seed-mediated, surfactant directed synthesis. An aspect ratio of ~4.5 was chosen to such that the surface plasmon resonant response matched the center wavelength used in our SD-OCT system (λ_{spr} = 840nm). An intravitreal injection of 2µl of the GNR solution was administered in one eye of eight healthy adult male C57Bl/6 mice; a sham intravitreal injection of 2µl of phosphate buffered saline (PBS) was administered to four additional mice. Raster 3D SD-OCT images of the retina were acquired before and after injection for at least one week using the same scanning protocol (1.5x1.5mm scan, 250x250x1024 pixels; Bioptigen, Inc, Durham, NC). Six mice (2 PBS, 4 GNR) were imaged for 8 days following injection and then sacrificed. The remaining mice were followed for 30 days. TEM was performed on the retinas of six mice and lenses of three mice.

Results:

An increase in intensity was seen in the vitreous in eyes injected with GNRs, and this was not observed in the eyes that received a PBS injection (Figure, a). GNRs were seen in the vitreous, decorating the hyaloid canal, adherent to the lens, within the lens and in microglia within the retina (Figure, b).

Conclusion:

GNRs injected into the vitreous stay in the vitreous for at least 30 days after injection. A change in backscattered intensity could be detected with SD-OCT while the GNRs were present.

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Drusen Area Calculations on Fundus and Corresponding *En-face* OCT Images: A Comparison

Peter Brennen, MD, Rick Bilonick, PhD, Thomas R. Friberg, MD

Introduction:

Semi-automated methods are available for the calculation of drusen area based on fundus images. Recently, several groups have used 3D SDOCT data for drusen volume and area calculations. We compared standard fundus photographs (FP) with *en-face* OCT images of drusen using a semi-automated drusen detection technique.

Purpose:

To identify differences between two drusen area calculation methods, one using fundus photography data, the other using OCT data.

Methods:

We retrospectively identified patients with dry macular degeneration who had 3D SDOCT data (Cirrus HD-OCT, Carl Zeiss Meditec, Dublin, CA) and fundus photography on the same day. An *en-face* OCT image was generated to represent drusen distribution in the macula by sampling along the Z axis above the RPE using the Advanced Visualization tool. Inclusion of low reflectivity outer plexiform layer A scan data provided contrast to high reflectivity drusen and RPE data for optimal images. OCT images and FPs were analyzed with the Drusen Analyzer software (F.O.R.T.H., Crete, Greece) to obtain the drusen area (mm^2) in a 3 mm diameter circle centered at the fovea. Two measurements were performed for each source data type.

Results:

En-face OCT and fundus image pairs were identified in and analyzed in ten eyes of 7 patients. Plotting of the differences versus the averages is presented in the Figure. When comparing OCT and FPs, a scale bias of 1.54 was demonstrated. Area measurements from FPs were larger than those obtained from OCT images. When

comparing imprecision, the relative imprecision of fundus photography as estimated by the method of maximum likelihood was much less, only 7% of the OCT imprecision.

Conclusions:
Our results from the sample eyes suggest poor agreement between OCT and fundus photographic image based drusen area assements. The higher relative imprecision of the OCT method suggests that further optimization is needed. Precise explanation of the scale bias is difficult as the absolute accuracy of the differing approaches had yet to be determined. Considering the presence of scale bias and degree of imprecision, it is likely that drusen volume calculations predicated on detection of drusen area based on FPs will differ from drusen volume calculated from OCT data alone.

NKG2A Expression Increases Susceptibility to Ocular HSV-1 Infection

Jared E. Knickelbein, PhD, Stephen A. Harvey, PhD, Robert L. Hendricks, PhD

Introduction:
Following herpes simplex virus type 1 (HSV-1) infection of the ocular surface, the virus sequentially replicates in the cornea, trigeminal ganglion (TG), and brain stem. Innate immune cells, including natural killer (NK) cells, help clear replicating HSV-1 during acute infection. NK cell effector functions are regulated by activating and inhibitory NK cell receptors. The inhibitory CD94/NKG2A heterodimer, which recognizes the non-classical MHC I molecule Qa-1 in mice (HLA-E in humans), serves to mitigate NK cell cytotoxicity.

Purpose:
To investigate the role of inhibitory NKG2A receptors in the pathogenesis of corneal HSV-1 infection

Methods:
DBA/2Ncr (NKG2A-expressing) and DBA/2J (NKG2A-deficient) mice were infected via the cornea with the RE strain of HSV-1 and were monitored for survival and development of herpes stromal keratitis (HSK) by clinical examination. At various times after infection, the indicated tissues were harvested and assayed by plaque assay for live virus titers, quantitative real-time PCR for cytokine transcripts, or flow cytometry for quantification and phenotyping of cellular populations.

Results:
DBA/2J mice that are incapable of expressing the NK cell inhibitory receptor NKG2A more efficiently control HSV-1 replication in the cornea, TG, and brain stem than their NKG2A-expressing DBA/2Ncr counterparts. The improved HSV-1 control was associated with reduced susceptibility to blinding HSK, a reduced rate of viral spread beyond the brain stem to the cerebellum and cerebral hemispheres, and a reduced

incidence of lethal encephalitis. The increased resistance of DBA/2J mice to HSV-1 was not related to impaired lymphocyte infiltration into infected ganglia, lymphocyte expression of granzyme B, or cytokine mRNA expression in infected tissues, but was related to reduced lytic granule exocytosis in NKG2A-expressing NK cells.

Conclusions:
Our data suggest that the inhibitory NKG2A receptor impairs lytic granule-mediated NK cell control of HSV-1 replication at the three checkpoints that are critical for limiting HSV-1-induced morbidity and mortality. Thus, blocking NKG2A in vivo may prove to be an effective therapy for HSV encephalitis.

An Evaluation of Objective and Subjective Quantitative Parameters at the Initial Visit to Predict Future Glaucomatous Visual Field Progression

Allison Ungar, Gadi Wollstein, MD, Hiroshi Ishikawa, MD, Rick Bilonick, PhD, Robert J. Noecker, MD, Juan Xu, PhD, Larry Kagemann, PhD, Joel S. Schuman, MD, FACS

Purpose:
To evaluate the Disc Damage Likelihood Scale (DDLS) and Vertical Cup-to-Disc Ratio (VCD) and quantitative objective parameters from Optical Coherence Tomography (OCT) and Confocal Scanning Laser Ophthalmoscopy (CSLO) in predicting future glaucomatous visual field (VF) progression.

Methods:
A prospective longitudinal analysis of 122 eyes of 70 subjects with ≥5 VF tests and single baseline measures from stereophotos (SP), OCT, and CSLO all acquired within 6 months of each other. DDLS and VCD were determined by the average independent opinion of 3 glaucoma experts. VF progression was defined by the glaucoma progression analysis (GPA) and visual field index (VFI) slope significance provided by the machine. Generalized estimating equation (GEE) models were used to determine the predictive ability of each parameter.

Results:
Median length of follow-up time was 4.0 years (range 1.5 to 5.7 years). Fifteen eyes progressed based on GPA, 20 by VFI, and 10 by both GPA and VFI. Baseline parameters from SP (VCD, DDLS), OCT (global mean, superior quadrant, inferior quadrant, and 6 o'clock), and CSLO (cup shape measure, mean cup depth) were predictive of future glaucomatous VF progression. When comparing the single best parameter from the SP grading models, OCT models, and CSLO model, only the OCT baseline parameter was statistically significant in predicting either GPA or VFI

progression. Standardized data showed the OCT superior quadrant retinal nerve fiber layer to have the highest odds ratio to predict progression by both GPA and VFI.

Conclusions:
Baseline parameters from SP, OCT, and CSLO scans may be useful clinically to predict future glaucomatous VF progression.

Ethyl Pyruvate's Effects on the TGF-B1-Induced Expression of α-Smooth Muscle Actin and Fibronectin in Human Tenon's Capsule and Trabecular Meshwork Cells

Akshar Abbott, J. Chen, T. Stubbs, Nirmala SundarRaj, PhD, Joel S. Schuman, MD, FACS

Purpose:
Previously, we have shown that ethyl pyruvate (EP), a stable derivative of pyruvic acid, decreases fibrosis fibroblast transformation in corneal stromal cells. The purpose of this study is to examine whether EP also exhibits anti-fibrotic effects in human Tenon's capsule and trabecular meshwork (TM) cells.

Methods:
TM and Tenon's capsule cells derived from human donors eyes were grown in culture and were treated with 15mM and 17.5mM EP. The effect of EP on cell proliferation was analyzed by immunostaining for a proliferative nuclear antigen (Ki67).The expression of alpha-smooth muscle actin (SMA) and fibronectin was evaluated by immunocytochemical and western blot analyses. Total mRNA was extracted from the cells using the Qiagen Rneasy Mini Kit. Relative abundance of mRNAs encoding fibronectin and alpha-SMA was estimated by quantitative real time RT-PCR.

Results:
EP, at 15 and 17.5 mM concentrations, inhibited TGF-beta1-induced proliferation of both TM and Tenon’s capsule cells. This was evident from lack of significant increase in cell numbers and less than 1% Ki67 positive cells after 2-4 days in culture in the presence of EP. The expression of alpha-SMA and fibronectin in both cell types was downregulated by EP as evident from decreased immunostaining and from significant loss in the intensities of the immunoreactive bands of fibronectin and alpha-SMA in the western blots of the proteins extracted from the cells. Furthermore, the levels of mRNAs encoding these proteins were lower in the EP treated cells than in the control cells, indicating that EP downregulates the transcription of fibronectin and alpha-SMA.

Conclusions:

These findings suggest that EP exhibits anti-fibrotic effects on human Tenon's capsule and trabecular meshwork cells. Therefore, EP may have therapeutic potential as an agent for decreasing TGF-B1-induced fibrosis in these cells, which can occur following trabeculectomy surgery.

Regulation of Gastrokine-1, Uroplakin-1B and Uroplakin-3B Promoter Activities by KLF4, KLF5 and Oct1

Doreswamy Kenchegowda, PhD, Sudha Swamynathan, PhD, Shivalingappa Swamynathan, PhD

Purpose:
Previously, we found that the expression of gastrokine (GKN1), uroplakin (UPK)-1B and -3B, which help maintain the epithelial integrity, is downregulated in the *Klf4* conditional null (*Klf4*CN) cornea. In the present study, we have examined the role of KLF4 in epithelial barrier formation and characterized the regulation of GKN1, UPK1B and UPK3B promoter activities by KLF4, KLF5, and Oct1.

Methods:
Expression levels of GKN1, UPK1B and UPK3B in the wild type (WT) and *Klf4*CN corneas were compared by Q-RT-PCR. Influence of KLF4, KLF5 and Oct1 on GKN1, UPK1B and UPK3B promoter activities was tested by transient co-transfection assays in NCTC human keratinocytes. Anti-*Klf4* shRNA was used to suppress the expression of *Klf4*. Trans-epithelial electrical resistance (TEER) was used as a measure of barrier forming ability of human corneal epithelial (HCE) cells in culture.

Results:
Downregulation of GKN1, UPK1B and UPK3B in the *Klf4*CN corneas was confirmed by Q-RT-PCR. Activity of the -479/+16 bp GKN1 proximal promoter fragment was stimulated 2.6-, 28.4- and 2.8-fold respectively, by pCI-KLF4, pCI-KLF5 and pCI-Oct1. The -479/+16 bp GKN1 promoter activity was stimulated upon co-transfection with KLF4 and KLF5 (18.9-fold), or KLF4 and Oct1 (6.9-fold), or KLF5 and Oct1 (67.8-fold), or KLF4, KLF5 and Oct1 (24.2-fold). Activity of UPK1B and UPK3B promoter fragments of different lengths was stimulated by 10- to 50-fold upon co-transfection with pCI-KLF4. Anti-KLF4 shRNA treated HCE cells showed significant reduction in the TEER (26 to 65 Ω) compared to WT (428 Ω) and control shRNA treated cells (404 to 430 Ω).

Conclusions:
GKN1 proximal promoter activity is stimulated the most by KLF5, followed by Oct1 and KLF4. KLF5 and Oct1 have a synergistic effect on GKN1 promoter activity. Barrier forming ability of HCE cells is compromised upon downregulation of *Klf4*. Thus, KLF4, along with KLF5 and Oct1, contributes to the corneal epithelial integrity by regulating the expression of GKN1, UPK1B and UPK3B.

CD44V6 Mediates Migration of Corneal Stromal Fibroblasts

Xuan Li, PhD, Yiqin Du, PhD, Kira Lathrop, James Funderburgh, PhD

Purpose:
During wound healing keratocytes become activated and migrate into the wounded area resulting in corneal haze or permanent scarring. Previously we showed hyaluronan (HA) to be induced during keratocyte activation by transforming growth factor beta (TGFB). HA mediates expression of scar-associated extracellular matrix components and cell motility. In the current study we examined roles for CD44, the major cell surface receptor of HA, in the migration of stromal fibroblasts.

Methods:
Isoforms of CD44 were detected using RT-PCR and immunoblotting with isoform-specific antibodies. Migration was induced by scratch-wounding of confluent low-passage human corneal fibroblasts in the presence of inhibitors, 4-methylumbelliferone (4MU), 4-methylesculetin (4ME), function-blocking antibodies to CD44, or a peptide containing 10-amino acids of the CD44V6 splice form. Both rate and directionality of migration was quantified by individual cell-tracking using real time imaging of DiO stained live cells.

Results:
CD44 was rapidly upregulated in keratocytes by TGFB. All 10 alternately-spiced regions of CD44, V1-V10, were identified in bovine keratocyte mRNA. Inhibitors 4ME and 4MU markedly reduced the CD44 upregulation. These inhibitors or the presence of any of three CD44 function-blocking antibodies (IM7, BRIJ35, and V6-specific CD44 antibody) significantly reduced the rate of human corneal fibroblast migration. A peptide containing sequence from the CD44V6-region resulted in a loss of directional migration.

Conclusions:
CD44 function is important for keratocyte migration as it is in other cell types. We show for the first time that 4MU and 4ME prevent CD44 synthesis, and that CD44V6 spice

form is involved in mediating migration. HA is not present in normal corneal matrix, but may play an important role in cell signaling during healing, including mediation of both migration and matrix synthesis. The simultaneous upregulation of both HA and its receptor CD44 during differentiation of keratocytes to myofibroblasts seems likely to be essential for migration of the cells into the wound. Targeting this migration using inhibitors such as 4MU may be useful approach to prevent haze and scarring resulting from corneal wound healing.

The Optos Imaging System as an Ophthalmoscope: Fundus Assessment of Problem Eyes Where Conventional Examination Techniques Fail

Kristin Rarey, MD, Thomas R. Friberg, MD, Andrew W. Eller, MD

Purpose:
To investigate the utility of Optos P200 (Dunfermline, Scotland) imaging to evaluate the fundi of patient eyes where tiny pupils or media opacities made conventional ophthalmoscopy and biomicroscopy impossible to perform.

Methods:
In a 12-month retrospective case analysis, we reviewed 8 patients [12 eyes] with extremely limited or no views to the posterior segment due to very small pupil diameters, dense nuclear cataracts, extensive posterior synechiae, or other media opacities, alone or in combination. These eyes were unable to be satisfactorily examined by standard slit lamp biomicroscopy using any standard contact or hand-held lenses lenses, or by indirect ophthalmoscopy. The examinations were attempted by two retina specialists, each with more than 25 years experience as subspecialists. The patients were then sent for imaging using the Optos P200 device and B-scan ultrasonography if no fundus images could be obtained

Results:
Of the 12 eyes that were imaged in our study, the cause of the poor view to the posterior pole included extensive posterior synechiae (3 eyes), anterior or posterior lenticular capsule fibrosis (3 eyes), pupils less than 3mm in diameter (5 eyes), and dense nuclear sclerotic cataract (3 eyes). Imaging quality was deemed adequate to make a clinically relevant assessment in all cases except 3 eyes, two of which had pupils less than 2 mm in diameter, and one that had dense vitritis. In all, Optos P200 images were clinically useful and allowed appropriate diagnoses to be made in 9/12 cases studied. Diagnoses included uveitis/parsplanitis, proliferative diabetic retinopathy, vasculoproliferative tumor, necrotizing retinitis, Vogt-Koyanagi-Harada Syndrome, and fungal endophthalmitis.

Conclusion:
In many eyes with tiny pupils or media opacities, retinal examinations using the Optos P200 ultrawidefield imaging system can often be conducted where conventional examination techniques yield virtually no view of the retina. Such eyes would typically be deemed impossible to examine with light, and would be assessed with B scan ultrasonography only. In this setting, the Optos P200 system can be considered a specialized ophthalmoscope capable of documenting the retina findings very quickly.

Analysis of Morphological and Visual Acuity in AMD Patients to Identify Early Indicators of the Progression of Dry to Wet AMD

Veeral Shah, MD, PhD, Peter Brennen, MD, Rick Bilonick, PhD, Thomas Friberg, MD

Purpose:
Early intervention with anti- VEGF drugs can significantly improve vision loss inneovascular (wet) Age-related Macular Degeneration (AMD), suggesting the need for early surveillance to identify eyes at risk for dry to wet AMD progression. Few studies have examined early detection of neovascular AMD with respects to morphological changes and characteristic vision loss. In this study, we examined the likelihood of developing neovascular AMD, in particular Choroidal Neovascularization (CNV), by evaluatingmacular fundus photos and documented visual acuity changes of AMD patients collected over time.

Methods:
A semi-automated analysis of fundus photos from 513 pt (834 eyes), were longitudinally followed from the Age-Related Eye Disease Study (AREDS) and Prophylactic Treatment of Age-related Macular Degeneration (PTAMD). Images were assessed for morphological abnormalities including macula pigmentation, drusen area and distribution. Patients' factors also included in our assessment was age, development of CNV in fellow eye, and ETDRS best –corrected visual acuity.

Results:
33 of the 513 (6.4%) subjects developed CNV, with visual acuity and aging variables showing significant correlation. A three- letter drop in ETDRS visual acuity predicted a 50% increase in theprobability for CNV develop in that eye (1.50, $p< 0.0001$). Statistical analysis showed that for single –letter drop in visual acuity had a 12% (1.12, $p<0.001$) increase in the possibility of CNV event, and comparably there was a 0.3% increase each day the eye was in the study. There was no difference in this effect if data from the 2 study groups were assessed independently.

Conclusion:
Our analysis suggests that decrease in visual acuity over time increases the possibility of CNV event. The likelihood of a CNV event also increased as AMD patients mature with age. Our data analysis of morphological abnormalities showed absence of early detection, which may be due to the infrequence of observable events.

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