A compilation of investigations made by Cleveland Clinic Cole Eye Institute physicians, research scientists and distinguished colleagues.

The abstracts presented were published in selected journals or presented at selected national meetings in 2003.
Dear Colleague:

I am pleased to share with you our 2003 Cleveland Clinic Cole Eye Institute abstracts. These abstracts reflect the exciting work being done in ophthalmology and bring new insights to a rapidly advancing specialty.

The Cole Eye Institute offers a wide range of adult and pediatric ophthalmologic services. We provide many highly specialized tertiary care services, including vitreoretinal surgery, corneal transplantation, anterior segment reconstruction, glaucoma implants, neuro-ophthalmology, ocular inflammatory diseases, and ophthalmic oncology, among others.

Clinical and basic ophthalmology research at the Cole Eye Institute is carried out by our professional staff of 60 physicians and scientists, who offer expertise in every ophthalmic subspecialty area.

We welcome your interest in our ophthalmology research and clinical studies. If you would like additional information, please call 216/444-2020 or visit our Web site at www.clevelandclinic.org/eye.

Sincerely,

Hilel Lewis, M.D.
Director, Cole Eye Institute
Chairman, Division of Ophthalmology
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Steven Wilson, Marcello Netto, R. Ambrosio, Jr.

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### High-Speed Optical Coherence Tomography of Anterior Segment Surgical Anatomy and Pathology

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Cornea
Automated Anterior Chamber Biometry with High-speed Optical Coherence Tomography

Y. Li, Maria Regina Chalita, J. Goldsmith, V. Westphal, B.A. Bower, R. Shekhar, A.M. Rollins, J.A. Izatt, David Huang

PURPOSE: Accurate sizing of angle-supported anterior chamber intraocular lens (AC-IOL) is crucial in preventing complications. To accurately measure AC width and other dimensions, we developed a high-speed optical coherence tomography (OCT) system and automated image processing.

METHODS: The OCT prototype operated at 1.3 micron wavelength and was capable of 8 images/sec at 500 axial scans/image. Scan dimensions are 16mm wide and 6mm deep (in air). The OCT scanner was adapted to a slit-lamp with video camera. Horizontal cross-sectional OCT images of the anterior segment was obtained. Each of the 40 eyes from 20 healthy volunteers was scanned 3 times. A computer algorithm was developed to measure angle-to-angle AC width, AC depth, and lens vault. These measurements were also obtained manually by 3 ophthalmologist using computer calipers on screen displays of OCT images.

RESULTS: The computer algorithm successfully measured AC diameter and AC depth from all 120 OCT images. The difference between computer and human measurements was 0.13±0.14 mm (mean±SD) for AC width, 0.04±0.04 mm for AC depth, and 0.08±0.06mm for lens vault. The image-to-image reproducibility of computer measurements (pooled SD) is 0.11 mm for AC width, 0.04mm for AC depth, and 0.07mm for lens vault. The image-to-image reproducibility of human measurement was 0.13 mm for AC width, 0.05 mm for AC depth, and 0.06mm for lens vault. The agreement between the human raters (inter-rater SD by analysis of variance) is 0.30 mm for AC width, 0.05 mm for AC depth, and 0.08mm for lens vault.

CONCLUSION: Due to its longer wavelength, the OCT system was able to penetrate and image the angles. The speed was sufficient high for reproducible AC width measurement. The automated computer algorithm agrees well with human raters. The use of a computer measurement algorithm avoids the relatively large disagreement between human raters for AC width. The use of OCT to directly measure AC width may improve the fitting of AC-IOL and avoid complications such as IOL dislocation and pupil ovalization.
Corneal Cells: Chatty in Development, Homeostasis, Wound Healing, and Disease

Steven Wilson, Marcello Netto, R. Ambrosio, Jr.

PURPOSE: To provide an overview of cell-cell interactions in the cornea that have a critical role in corneal development, homeostasis, wound healing, and disease.

METHODS: Review

RESULTS: Cell-cell interactions make critical contributions to development, homeostasis, and wound healing in the cornea. Many of these interactions are mediated by cytokines, growth factors, and chemokines. The best characterized are stromal-epithelial interactions between epithelial cells and stromal cells such as kerocytes, keratoblasts, and myofibroblasts. However, interactions also occur between corneal nerves and epithelial cells and between corneal cells (epithelial cells and stromal cells) and corneal immune cells. Although investigations are limited, it is likely that there are interactions between corneal endothelial cells and kerocytes in the posterior stroma.

CONCLUSIONS: Cellular communications in the cornea are critical during development, homeostasis, and wound healing. Disorders of cellular communication likely contribute to many corneal diseases.
Dual-Wavelength Optical Coherence Tomography for Hydration Analysis
Sung W. Jeon, Mark A. Shure, Andrew M. Rollins, David Huang

BACKGROUND: Optical Coherence Tomography (OCT) provides non-invasive, high-resolution, cross-sectional imaging of internal structures. By combining two light sources, one at 1294 nm (where absorption losses are small) and one at 1410 nm (near the water absorption peak), OCT can be an effective tool for deriving hydration level within tissues. An important application of this technique is quantitative measurement of how corneal hydration affects the results of laser in-situ keratomileusis (LASIK) and photorefractive keratectomy (PRK). OCT hydration measurement may also be used for quantifying (1) variation of hydration in population (2) variation in hydration as a function of corneal depth (3) corneal hydration as a function of menstrual cycle.

METHODS: We measured the water absorption coefficient and hydration level in a phantom containing a carefully controlled fluid mixture and in human cornea in vitro with a dual-wavelength OCT technique. The phantom was prepared from a mixture of water and deuterium oxide with various Intralipid levels to simulate turbid tissue conditions. Deuterium oxide, also known as heavy water, has similar optical properties to water other than negligible absorption within used wavelengths. Two OCT signals were recorded simultaneously with two light sources whose spectra are centered within (1410 nm) and outside the water absorption (1294 nm) band. Absorption coefficient and hydration were calculated from the logarithmic intensity ratio of the two signals.

RESULTS: The result showed that a strong correlation exists between the measured water absorption coefficient and the actual water content in the phantom. With Intralipid in the fluid mixture, the predicted absorption coefficient deviated from our expectation. Cornea hydration predicted from Dual-Wavelength OCT matched with the hydration level from the standard weight method.

CONCLUSION: Preliminary results suggest the potential of dual-wavelength OCT to fill a valuable niche in hydration level measurement.
High-Speed Optical Coherence Tomography of Anterior Segment Surgical Anatomy and Pathology

David Huang, Maria Regina Chalita, Yan Li, Careen Y. Lowder, David M. Meisler, Andrew M. Rollins, Joseph A. Izatt

PURPOSE: To use a high-speed corneal and anterior segment optical coherence tomography (CAS-OCT) system to image ocular pathologies and surgical anatomy.

METHODS: A high-speed (4000 a-scan/sec) wide-field (16 mm) CAS-OCT system was developed. It uses a longer wavelength (1.3 microns) compared to retinal OCT systems (0.8 microns). OCT scans were performed on 11 eyes with anatomic features of interest.

RESULTS: OCT of post-surgical cornea (LASIK, penetrating keratoplasty), trabeculectomy bleb, anterior chamber intraocular lens (IOL), iris masses and cataract were obtained. Full-thickness imaging of sclera, angle and iris was possible. No appreciable motion artifact was noted at 8 frames/sec. The entire LASIK flap could be fully visualized. In keratectasia, OCT showed relative corneal thinning in the area of steepening. Causative factor such as inadequate residual posterior stromal thickness and excessive flap thickness could be quantitatively assessed. The longer 1.3-micron wavelength allowed the angle recesses to be visualized. The recess-to-recess anterior chamber width could be directly measured, along with other parameters such as the anterior chamber depth and crystalline lens vault. In trabeculectomy images, the sclerotomy site could be visualized as well as the whole bleb anatomy. The anterior chamber IOL were seen and the footplates position were recorded. Iris and ciliary body masses could be precisely delineated and accurately measured.

CONCLUSION: The CAS-OCT prototype allowed non-contact visualization and measurement of corneal and anterior segment pathologies and surgical anatomy. The high speed allows quantitative measurements of relevant biometric dimensions. The longer wavelength (1.3-micron) allows greater penetration through highly scattering tissue such as limbus and sclera.
Inhibition of Nitric Oxide Synthesis in Corneas in Corneal Storage Media

David M. Meisler, T. Koeck, J.T. Connor, K.S. Aulak, Bennie H. Jeng, D.J. Stuehr

PURPOSE: To determine if nitric oxide synthesis by human corneas can be inhibited during corneal storage.

METHODS: 1.0 mM of monomethyl-L-arginine (LMMA), a nitric oxide synthase inhibitor, was added to four chambers of Optisol GS corneal storage media, each containing a viable human corneal button. Each chamber was sampled (0.4 ml) for baseline measurements and at one-day intervals for 21 days. Four chambers of Optisol GS corneal storage media, each containing companion corneas to the corneas exposed to LMMA served as controls and were sampled in tandem with the LMMA-containing chambers. An unused vial of Optisol GS corneal storage media served as a background media control. The total amount of nitrate and nitrite (stable breakdown products of nitric oxide) in each sample was determined using a spectrophotometric method based on the Greiss reaction. A polynomial random coefficients model, fitting Concentration on linear, quadratic, cubic, and square-roots terms times time in Days, was fit. The derivative of the fitted equation was also investigated to estimate rates of accumulation over time.

RESULTS: Data from the daily sampling of LMMA-containing media showed a statistically marked reduction in the rate of accumulation of nitrate and nitrite concentrations, as compared to controls (p<0.01), up until Day 10 when the rates became and remained equivalent. There was a marked reduction in the levels of nitrate and nitrite accumulation in the study chambers as compared to control chambers for each time point during the course of the study (p<0.01, e.g. 1.03 µM 95% CI 0.92-1.16 µM at 10 days). There was also a substantial delay (3 days) in the rise of nitrate and nitrite concentration in the study group. Accumulation rates were more than 0.15 µM/day higher for the first three days in the media without LMMA. No nitrate and nitrite were detected in the background media control sample.

CONCLUSIONS: The progressive increase in nitrate and nitrite accumulation in corneal storage media can be delayed and blunted by the addition of a nitric oxide synthase inhibitor. Given the toxic free radical properties of nitric oxide, corneas in storage awaiting transplantation may benefit from having a nitric oxide synthase inhibitor added to storage media.
Inhibition of Nitric Oxide Synthesis in Corneas in Storage Media


PURPOSE: The nitrate/nitrite content in storage media was determined after nitric oxide synthase inhibition by adding 400 microl of 100 mm N(G)-monomethyl-l-arginine (LMMA) to four chambers of Optisol GS corneal storage media, each containing one viable human cornea. The companion corneas in storage media without LMMA served as controls.

METHODS: Four hundred microlitre aliquots obtained at baseline (day 0) and at one-day intervals for 20 more days for both groups were analyzed for nitrate and nitrite (breakdown products of nitric oxide) concentration levels using a spectrophotometric method based on the Greiss reaction.

RESULTS: Average nitrate/nitrite concentrations, statistically analyzed using a polynomial random coefficients model, showed a statistically significant marked reduction in the levels of nitrate and nitrite accumulation in the study chambers as compared to control chambers for days 1-20 (P < 0.001) There was also a reduction in the accumulation rate of nitrate and nitrite concentrations, as compared to controls (P < 0.05) until around day 8 when the differences in rates were no longer statistically significant.

CONCLUSION: The progressive increase in nitrate and nitrite accumulation in corneal storage media can be blunted by the addition of a nitric oxide synthase inhibitor. Given the toxic free radical properties of nitric oxide, corneas in storage awaiting transplantation may benefit from having a nitric oxide synthase inhibitor added to storage media.
Modeling the Thermal Response of the Retina during Corneal OCT Scans
Mark A. Shure, Sung W. Jeon, David Huang

BACKGROUND: The maximum permissible exposure levels quoted in the ANSI Z136.1-2000 Standard for Safe Use of Lasers do not specifically address the optical beam configuration employed in the corneal anterior chamber optical coherence tomography (CAS OCT) system used at Cole Eye Institute for clinical trials. Therefore, we undertook a theoretical study in order to understand the risks of thermal damage from our instrument. This is also part of our study on the safety of high speed corneal and anterior segment OCT, which use higher power to enable high speed scans.

METHODS: Absorption in the ocular media ahead of the retina was estimated from published values. The beam spot size on the retina was computed by tracing rays through an optical model which included the optical elements of the CAS OCT system as well as the optical properties of the human eye. A dynamic heat transfer model of the retina was developed in order to compute the temperature distribution as a function of position and time.

RESULT: We present the equilibrium temperature distribution predicted for standard clinical use and for the worst-case scenario. The range of results is summarized by computing the maximum temperature increase for a wide range of retinal spot sizes.

CONCLUSION: As a result of our thermal model, we found that even under the most pessimistic conditions (a diffraction-limited spot held stationary on the retina for a second or more), our thermal model predicts only a 1.9°C rise in temperature in the choroid. None of these heating results is high enough to cause thermal damage to the retina.
Repeat Penetrating Keratoplasty

Roger H. S. Langston

PURPOSE: To compare results of initial and repeat penetrating corneal transplants.

METHODS: A chart review of 44 repeat penetrating corneal transplants performed between 1989 and 1999 compared results in the initial and repeat grafts with respect to initial diagnosis, cause of failure, associated ocular conditions, visual acuity and refractive error.

RESULTS: First regrafts were found to have fourteen percent increase in rate of failure compared to initial grafts, two lines less best corrected spectacle acuity on average and no increase in induced refractive error, including astigmatism. The presence of anterior chamber implants, glaucoma, and especially glaucoma setons, increased the risk of graft failure.

CONCLUSION: Repeat penetrating corneal transplants have similar refractive results to initial grafts but lose on average two lines of acuity and are more likely to fail.
Group Index of Human Cornea at 1.3\(\mu m\) Wavelength Obtained In Vitro by Optical Low-Coherence Reflectometry

Roger C. Lin, Mark A. Shure, Andrew M. Rollins, Joseph A. Izatt and David Huang

PURPOSE: The group index of the cornea, rather than the phase refractive index, is required for thickness calculations with optical coherence tomography. Recent advances with high-speed optical coherence tomography at 1.3 \(\mu m\) make index measurement at this wavelength of great interest.

METHODS: Group indices of three human corneas from an eye bank were measured in vitro with optical coherence domain reflectometry. Measurements were made in a calibrated cuvette filled with a preservation medium to maintain proper corneal hydration. Group indices were calculated from the optical path lengths measured.

RESULTS: The corneal group index was 1.389 ± 0.004 (average ± standard deviation). The average group index of a balanced salt solution, an approximation to aqueous humor, was 1.343 ± 0.001.
In Vivo Real Time Imaging of Bone Marrow-Derived Inflammatory Cells Migration into the Cornea During Lipopolysaccharide Induced Keratitis

M. Roche, A. Hsia, L. Van Parijs, Victor L. Perez

PURPOSE: To establish an in vivo technique to visualize in real time the migration of bone marrow-derived inflammatory cells into the cornea during the induction of keratitis.

METHODS: GFP bone marrow chimera mice were generated to track the migration of inflammatory cells into the cornea. Briefly, bone marrow-derived stem cells were first harvested from the tibia and fibula of mice treated with 5-FU. After expansion in vitro, bone marrow stem cells were infected with a retroviral vector expressing GFP. GFP positive transfected cells were then transplanted into a lethally irradiated mouse and reconstitution was confirmed by flow cytometry analysis. Keratitis was induced by intrastromal injection of LPS (2ug) in the cornea of GFP chimera mice. Migration pattern of GFP cells into the cornea was evaluated at different time points by in vivo real time imaging, using fluorescent microscopy and digital camera with time elapse software. Immunohistochemical analysis was done ex-vivo to identify infiltrating GFP positive inflammatory cells.

RESULTS: GFP positive cells were detected in the peripheral blood, thymus, spleen and bone marrow of GFP bone marrow chimera mice by flow cytometry. Bone marrow-derived GFP cells were present in the corneal limbus prior to treatment. In vivo microscopy showed migration of GFP positive cells from the limbus into the cornea as early as 30 minutes after intrastromal LPS injection. Time elapse analysis revealed a dynamic and random pattern of GFP positive cells migration migrating from limbus to limbus across the cornea. Immunohistochemical staining of corneal specimens demonstrated the presence of neutrophils and macrophages in the cornea.

CONCLUSION: Real time visualization of inflammatory cells migration into the cornea can be characterized and defined accomplished in GFP chimera mice using time elapse in vivo imaging. Bone marrow-derived inflammatory cells reside in the limbus and rapidly migrate across the cornea in response to LPS. We believe that real time imaging in the cornea will allow us to define mechanisms involved in the recruitment of neutrophils and macrophages into a site of inflammations of inflammation.
Expression of Soluble FAS Ligand in the Cornea Inhibits Lipopolysaccharide Induced Keratitis

Victor L Perez, M. Gregory, A. Marshak-Rothstein, B. Ksander

PURPOSE: We have previously shown that the selective expression of soluble Fas Ligand (sFasL) in the cornea does not induce inflammation and blocks the activation of neutrophils. We hypothesize that sFasL effectively blocks the development of innate immunity and therefore will prevent destructive corneal inflammation associated with keratitis. To test this, we examined the effects of sFasL in a model of lipopolysaccharide (LPS) induced keratitis.

METHODS: sFasL was expressed in the corneal stroma of C57BL/6 mice by adenoviral transduction, using intra-stromal injection of adenoviral vectors. The adenoviral vector contained a gene encoding the soluble-only form of FasL and a GFP marker gene, both under the control of a CMV promoter. Gene expression was confirmed by measuring GFP expression in the cornea with fluorescent microscopy and by western blot. To induce keratitis, LPS intrastromal injections (4ug) were performed 24 hrs after gene transduction, and corneal inflammation was monitored by slit-lamp examination and histological analysis.

RESULTS: Intra-stromal injection of sFasL or control vector alone resulted in GFP expression for 30 days, without any evidence of inflammation. Intrastromal injection of LPS into corneas transduced with the control adenoviral vector developed keratitis at 3-5 days, followed by corneal scarring and neovascularization after 10 days. By contrast, corneal expression of sFasL effectively prevented LPS induced corneal keratitis, scaring, and neovascularization. Histological analysis of corneas with LPS induced keratitis revealed a vigorous infiltration of neutrophils, which was reduced in corneas treated with sFasL.

CONCLUSION: Corneas expressing sFasL are protected against LPS induced inflammation and scarring. This is the first demonstration of the role of sFasL in regulating in vivo innate immune responses in a model of inflammation induced by LPS signaling. Furthermore, it suggests the potential use of sFasL in treating neutrophil mediated keratitis.
Section 2:

Glaucoma
Measurement of Retinal Ganglion Cell Layer and Inner Plexiform Layer Thickness with Optical Coherence Tomography

O. Tan, Yan Li, David Huang

PURPOSE: To investigate direct measurement of the retinal ganglion cell layer (GCL) and inner plexiform layer (IPL) thickness with the commercial available optical coherence tomography (OCT).

METHODS: The OCT3 system (Carl Zeiss, OCT3000, Dublin, CA) was capable of acquiring 400 axial-scans (A-scan) per second with axial resolution of 10 micron in tissue. 2, 2.5, 3, 3.5, and 4 mm diameter circular retinal scans (centered at fovea) were obtained from normal volunteers with an OCT3 system. Each circular scan comprised 256 A-scans. An automatic computer algorithm was developed to process the resulting OCT images. Firstly, A-scans in each image were aligned according to the vitreo-retinal boundary. Second, a Gaussian low-pass filtering was used to smooth the image and suppress the speckle noise. Then a progressive segmentation was performed to detect the boundaries based on the gradient of the filtered OCT image.

RESULTS: The thickness of the retinal layers were measured from each of the images and statistical analysis was performed. As expected, the GCL thickness decreases with increasing distance from the fovea. The thickness of the IPL is relatively constant in the area centralis. The SD of the combined GCL+IPL thickness is smaller (3-5 microns) than that of the GCL alone (4-6 microns).

CONCLUSIONS: This is the first demonstration of the direct GCL and IPL thickness measurements on a clinical OCT3 system. The only other report of segmentation of these retinal sublayers are based on images from an ultrahigh resolution (1-3 microns FWHM) OCT system that uses femtosecond laser light source, which is too bulky and expensive for routine clinical use. We have achieved the same desirable end result by image processing on moderately high-resolution images.
Comparison of Optical Coherence Tomography and Ultrasound Biomicroscopy for Detection of Narrow Anterior Chamber Angles

Scott D. Smith, Jason A. Goldsmith, Sunita Radhakrishnan, Volker Westphal, David Huang, David K. Dueker, Andrew M. Rollins, Joseph A. Izatt

PURPOSE: To assess the accuracy of classification of narrow anterior chamber (AC) angles using quantitative imaging by optical coherence tomography (OCT) and ultrasound biomicroscopy (UBM).

METHODS: A high-speed (4000 axial scans/sec) anterior segment OCT prototype was developed using a 1.3 mm light source. 17 normal subjects (17 eyes) and 7 subjects (14 eyes) with narrow angle glaucoma were enrolled in this prospective institutional study. All subjects underwent gonioscopy, OCT and UBM. Quantitative AC angle parameters were measured from OCT and UBM images using proprietary processing software.

MAIN OUTCOME MEASURES: Specificity and sensitivity in identifying narrow angles with image-derived AC angle parameters.

RESULTS: Eight of 31 eyes were classified as having narrow angles (Shaffer grade ≤1 in all quadrants). A strong correlation was found between gonioscopy grade and angle opening distance (AOD), angle recess area (ARA), and a newly defined parameter, the trabecular iris space area (TISA) measured both by OCT and UBM (all p-values <0.0005). Both OCT and UBM showed excellent performance in identifying eyes with narrow angles, with areas under the receiver operating characteristic (ROC) curves for these parameters in the range of 0.91 to 0.99. The best parameters were AOD, ARA and TISA (sensitivity=100%, specificity=95.7%) as measured by OCT.

CONCLUSIONS: Angle grading with both OCT and UBM agreed well with gonioscopy. OCT performed slightly better, was easier to use and did not require contact with the eye. OCT is a promising method for screening individuals at risk for narrow angle glaucoma.
The Prevalence of Glaucoma in an Elderly Population in Saudi Arabia
Scott D. Smith, Ibrahim Al-Jadaan, Monzer H. Jabak, Ali Al-Rajhi, Abdulrazzaq Al-Saif

PURPOSE: To determine the prevalence of glaucoma by mechanistic subtype in a population-based sample of elderly Saudis, age 60 years and older.

METHODS: A population-based census from each of two community health centers in the Al Kharj region of Saudi Arabia (approximately 100 km outside the Riyadh metropolitan area) was obtained, and a sample of 785 Saudi citizens age 60 years and older was selected at random. Comprehensive ophthalmic examination including slit lamp examination and gonioscopy by an ophthalmologist was performed for each participating subject. Patients in whom suspicion of glaucoma was present based upon tonometry and optic disk evaluation were sent for visual field testing with the Dicon LD400 40 point threshold-related suprathreshold screening exam with quantification of missed points. Final classification of glaucoma status was made by two trained glaucoma specialists and classified subjects as normal, possible, probable, or definite glaucoma, and a glaucoma mechanistic subtype was assigned for each patient. Disagreements in classification were reconciled by joint review of study charts when necessary.

RESULTS: Of the 785 subjects in the sample, a total of 565 (72.0%) agreed to participate in the survey. Fifteen subjects were examined in their home after refusing examination in the community health center. Each decade of increasing age was associated with an odds ratio for having probable or definite glaucoma of 1.49 (95% CI [1.05, 2.12], p=0.03). The median IOP of subjects with glaucoma was 27 mmHg, compared to 17 mmHg among the remaining subjects (Mann-Whitney, p=0.0001) The most common glaucoma subtypes were PNAG and POAG, with nearly equal prevalence with prevalence of 3.9% and 3.7%, respectively. Pseudoexfoliation glaucoma was also common with a prevalence of 2.0%. Among subjects without probable or definite glaucoma, the presence of occludable angles was 19% (95% C.I. [15.6% to 22.0%].

CONCLUSIONS: There is a very high overall prevalence of glaucoma in the elderly population of Saudi Arabia. Subjects initially refusing examination were older and more likely to have glaucoma, suggesting that the true glaucoma prevalence in this population may be higher than our reported estimate. Primary narrow-angle glaucoma is common, with prevalence approaching that reported in east Asian populations. Primary open-angle is common, with prevalence intermediate to that reported in other racial groups of comparable age. The high prevalence of primary narrow-angle glaucoma and occludable angles in Saudi Arabia indicates the need to develop effective methods of population-based screening and prevention in that region.
Proteomic Approaches to the Etiology of Glaucoma
Sanjoy K. Bhattacharya, Bruce Levison, Karen A. West, Edward J. Rockwood, John W. Crabb

PURPOSE: Increased intraocular pressure from resistance to aqueous humor outflow through the trabecular meshwork may influence the onset and progression of glaucoma. Our goal is to identify proteins and protein modifications in the trabecular meshwork associated with the pathogenic mechanisms of glaucoma.

METHODS: Trabecular meshwork tissue was collected from normal cadavers (n = 6) and from glaucoma patients undergoing trabeculectomy (n = 6). Proteomic analyses of trabecular meshwork from normal and glaucomatous donors were pursued using LC-MS/MS and bioinformatic methods. To identify possible oxidative protein modification, Western analyses were performed with antibodies to carboxyethylpyrrole (CEP, derived from docosahexanate-containing lipids), hydroxynoneal (HNE, derived from linoleate- and arachidonate-containing lipids) and argpyrimidine (an advanced glycation end product or AGEs).

RESULTS: Proteomic analyses of trabecular meshwork have so far identified 377 proteins, including 249 proteins from normal donor tissues, 57 proteins from glaucomatous donor tissue and 71 proteins common to both. Proteins known to be present in the trabecular meshwork and found in this preliminary study include TIGR or myocilin, myosin, apolipoprotein E, optimedin, laminin, decorin, and collagens I, IV and XVIII. Myocilin was observed only in glaucomatous tissue. Caspase-14, thrombin, TIMP-3, antithrombin, antitrypsin and several chaperons have so far only been observed in normal donor tissues. From comparative Western analysis, HNE immunoreactivity has only been found associated with glaucomatous trabecular meshwork.

CONCLUSIONS: We anticipate that further proteomic analyses of trabecular meshwork will lead to insights into glaucoma disease mechanisms, potential drug targets and therapeutic strategies.
Section 3:

Neuro-Ophthalmology
Ocular Ethambutol Toxicity
Alex Melamud, Gregory S. Kosmorsky, Michael S. Lee

PURPOSE: Ethambutol is an antimicrobial agent used frequently to treat tuberculosis. The most commonly recognized toxic effect of ethambutol is optic neuropathy, which generally is considered uncommon and reversible in medical literature.

METHODS: We describe a 43-year-old man who developed signs and symptoms of bilateral optic neuropathy during treatment with ethambutol.

RESULTS AND CONCLUSIONS: This case and a review of the literature show the severe and unpredictable nature of ethambutol toxicity and its potential for irreversible vision loss despite careful ophthalmologic monitoring.
Choroidal Neovascularization Associated with Meningioma
Michael S. Lee, S. Lessell

PURPOSE: To report the occurrence of three patients with choroidal neovascularization (CNV) associated with a meningioma involving the ipsilateral optic nerve. Numerous causes of CNV exist including chronic disc edema and choroidal tumors, but the underlying pathophysiology involves a break in Bruch’s membrane. A large review of meningiomas estimated 3.7% of 477 cases with intraocular invasion. Other authors have presented histopathologic evidence of clinically inapparent intraocular invasion by meningiomas.

METHODS: Retrospective chart review of three patients presenting to a tertiary care neuro-ophthalmology unit.

RESULTS: Three patients developed evidence of retinal elevation with fluorescein angiographic leakage consistent with CNV. MRI revealed enhancing lesions involving the ipsilateral optic nerve consistent with meningioma. None of the patients had evidence of previously reported causes of CNV.

CONCLUSION: Patients with meningiomas that involve the optic nerve may develop ipsilateral choroidal neovascularization in the absence of chronic disc edema. The presumed mechanism is intraocular tumor invasion sufficient to disrupt Bruch’s membrane, but below the threshold of clinical observation.
Ehrlichiosis Optic Neuritis

Michael S. Lee, S. Lessell

PURPOSE: To describe a case of ehrlichiosis optic neuritis. Design: Single observational case report.

METHODS: A 41-year-old woman with symptoms and clinical and imaging signs consistent with optic neuritis presented to a tertiary care academic center for comprehensive neuro-ophthalmic evaluation. Main outcome measures included preoptic and postoptic neuritis polyvalent ehrlichiosis titers and magnetic resonance imaging (MRI) of orbits with gadolinium.

RESULTS: Ehrlichiosis titers drawn 11 days before onset of eye symptoms were negative. Titers drawn 7 days after symptoms began were positive. The optic nerve enhanced with gadolinium on MRI.

CONCLUSIONS: Ehrlichiosis can cause optic neuritis and should be considered in patients with optic neuritis after a febrile, flu-like illness in an endemic area.
Laser Pointer Visual Field Testing: A Sensitive Screening Technique
Michael S. Lee, L.J. Balcer, N.J. Volpe, G.T. Liu, G.S. Ying, S.L. Galetta

PURPOSE: Sensitivity of confrontation visual field (CVF) screening is low unless defects are significant. We compared the sensitivity of laser pointer visual field screening (LVF) with conventional CVF for identifying eyes with abnormal automated perimetry.

METHODS: Ninety consecutive patients presenting for HVF prospectively underwent a masked comparison of CVF and LVF testing (175 eyes) from April to May 2000. LVF was performed using a laser pointer target projected onto a tangent screen. Points were tested in random fashion on either side of the vertical and horizontal meridians, near central fixation, around the blind spot, and in each quadrant. Single and double simultaneous finger counting was used to test CVF.

RESULTS: LVF demonstrated significantly greater sensitivity as compared with CVF (73% versus 31%, P = 0.001) in identifying field defects found on HVF. Specificities for LVF and CVF were 82% and 99%, respectively. The average testing times per eye were 0.5 minute for CVF, 1.5 minutes for LVF, and 8.0 minutes for HVF.

CONCLUSIONS: In this cohort, laser visual field testing was significantly more sensitive than confrontation testing. It may represent an effective, time-efficient tool for visual field screening.
Section 4:

Oculoplasty
Transcaruncular Orbital Decompression for Dysthyroid Optic Neuropathy
Julian D. Perry, Anish Kadakia, Jill A. Foster

PURPOSE: To determine the efficacy of transcaruncular approach orbital apex decompression for treatment of dysthyroid optic neuropathy.

METHODS: In this retrospective noncomparative interventional case series, charts for all patients undergoing orbital decompression surgery for dysthyroid optic neuropathy performed by one author between October 1999 and September 2001 were included in the study. Primary outcome measures included visual acuity, static perimetry, pupillary testing, and color plate testing before and after surgery. Records were also reviewed for changes in extraocular motility and proptosis after surgery and for surgical complications.

RESULTS: Sixteen consecutive patients (6 unilateral, 10 bilateral, for a total of 26 cases) underwent orbital apex decompression for dysthyroid optic neuropathy through a transcaruncular approach. In each orbit, the optic neuropathy was refractory to oral corticosteroid therapy. Preoperative visual acuity remained stable or improved in each case. Preoperative Humphrey visual field testing revealed an average mean deviation of -10.3±6.5 (range, +0.76 to -25.45). Average postoperative mean deviation was -2.79±2.4 (range, +0.94 to -9.82). Before surgery, 7 of 23 eyes (30%) had full color plates. After surgery, 22 of 23 eyes (96%) had full color plates. Follow-up ranged from 2 to 26 months (mean, 10 months). New-onset diplopia developed in 2 of 10 (20%) patients without preexisting diplopia.

CONCLUSION: Transcaruncular approach orbital apex decompression effectively treats dysthyroid optic neuropathy.
Simultaneous Ipsilateral Temporal Fossa and Orbital Dermoid Cysts

Julian D. Perry, Ralph Tuthill

PURPOSE: To describe a case of simultaneous dermoid cysts in the lateral orbit and temporal fossa.

METHODS: A 7-year-old boy with a lateral orbital mass and infratemporal fossa mass underwent computed tomography and surgical excision.

RESULTS: Intraoperatively, two distinct cystic lesions were identified. The orbital lesion extended just beneath the lateral rim. The temporal fossal lesion extended posteriorly along the temporal fossa. No bony defect in the lateral orbital wall was identified, and each distinct lesion was completely excised.

CONCLUSIONS: To our knowledge, this is the first reported case of multiple dermoid cysts in the orbital region. When imaging studies demonstrate separate cystic lesions and do not reveal a bony defect in the lateral orbital wall, multiple lesions should be suspected.
Section 5:

Ophthalmic Genetics
Thirty Year Clinical Follow-Up of a Patient with Novel RPE65 Mutations and Leber Congenital Amaurosis

K. Al-khayer, Stephanie Hagstrom, H. Zegarra, G. Pauer, Elias I. Traboulsi

PURPOSE: To report a North American family with heterozygous compound mutations in the RPE65 gene associated with Leber Congenital Amaurosis, and to present a long-term follow-up of the ocular findings in the proband.

METHODS: RPE65 mutation screening was performed on 30 patients with Leber Congenital Amaurosis (LCA) using PCR amplification of the 14 exons of RPE65, and search for sequence changes using SSCP and direct sequencing of abnormal bands. Ophthalmic examinations included visual acuity testing, ophthalmoscopy, color vision testing, dark-adapted threshold perimetry, and electroretinography.

RESULTS: The proband, a 35 year-old female carried two RPE65 mutations in a compound heterozygous fashion: a maternal K303X (A961T) nonsense mutation and a paternal Y431C (A1346G) missense mutation. She had severe visual deficits and an absence of rod and cone electroretinographic responses. Visual acuity of 20/60 and color recognition during early childhood declined over time to 20/100 OD and 20/50 OS with total absence of color recognition during the teenage years, and only 2/200 OD and 1/200 OS at the age of 30. She graduated from high school in regular classroom setting. Both parents had normal visual function and a sister carried one of the mutations.

CONCLUSIONS: The RPE65 mutations K303X and Y431C in compound heterozygous form cause progressive visual compromise that starts in childhood and advances to almost total visual loss by the fourth decade of life. The identification and characterization of the clinical course of patients with RPE65 mutations is important in preparation for future trials of gene therapy for retinal degeneration.
Clinical Correlation for Two Novel Mutations in the RPGR Gene for X-linked Retinitis Pigmentosa


PURPOSE: X-linked Retinitis Pigmentosa (XLRP) is a hereditary retinal degeneration that leads to the early onset of night blindness with progressive loss of peripheral vision and eventual legal blindness in most patients in later decades of life. We describe the clinical manifestations of two novel mutations in the RPGR gene in 3 patients. Mutations in RPGR have been associated with XLRP (RP3) and X-linked cone-rod dystrophy (COD1).

METHODS: Snellen visual acuity, ocular alignment, color vision assessment, intraocular pressure measurements and cycloplegic or manifest refractions were preformed, followed by complete slit lamp bimicroscopy and dilated fundus examination, electroretinograms, and Goldmann visual fields. Family ophthalmic histories were noted. Appropriate informed consent was obtained and DNA was extracted from whole blood samples. Coding regions plus ORF15 were polymerase chain reaction (PCR) amplified with intronic primers specific for the 19 exons and ORF15 of the RPGR gene. The amplified exon fragments underwent mutation analysis by standard direct sequencing techniques.

RESULTS: Patient #1, age 12 years, had a best corrected visual acuity (BCVA) of 20/25-, with onset of visual symptoms in the first decade; he had a mild to moderate myopic error of refraction, and carried a novel mutation in RPGR Exon 4 (333_336dup4). Patient #2, age 19 years, had BCVA of 20/40-, with onset of symptoms at age 13 years and a high myopic refractive error. Patient #3 was 27 years old; his BCVA was 20/200 with onset of night blindness at age 4 years; he was moderately to highly myopic. Both patient #2 and #3 had a novel mutation in RPGR ORF15 (ORF15+483_484delGA). Fundus appearance was typical of classic retinitis pigmentosa in all patients. Electroretinographic tracings were severely reduced or non-recordable in all patients.

CONCLUSION: There appears to be no significant difference in the clinical presentation between these two particular mutations in the RPGR gene that cause classic XLRP with myopia.
Mutation Screen in the Membrane-Type Frizzled-Related Protein (MFRP) Gene in 113 Patients with Inherited Retinal Degenerations

G.J. Pauer, Q.Xi, Elias I. Traboulsi, Stephanie Hagstrom

PURPOSE: MFRP is a member of the frizzled-related protein family and contains a cysteine-rich domain essential for Wnt binding and signaling. MFRP is highly expressed in the retinal pigment epithelial cells of the eye. A splice donor mutation in the mouse homolog of Mfrp is responsible for photoreceptor degeneration in the rd6 mouse, whose fundus is characterized by discrete dots distributed across the retina. We investigated MFRP as a candidate gene for a variety of retinal degenerations.

METHODS: To date, a partial screen (11 of 13 exons) for mutations in 47 unrelated patients with Stargardt’s macular dystrophy, 44 unrelated patients with Retinitis Pigmentosa (RP), and 22 unrelated patients with Leber Congenital Amaurosis (LCA) has been performed using exon-by-exon SSCP. Variant bands detected by SSCP were further analyzed by direct genomic sequencing.

RESULTS: Three missense sequence changes (Arg54Gly, Ile119Val, and Val136Met) were identified in MFRP. Arg54Gly was identified in one autosomal dominant Stargardt’s patient and Ile119Val was identified in one simplex Stargardt’s patient. The Val136Met missense change and three isocoding changes (Asp238Asp, Leu318Leu and Ala545Ala) were found in patients with all three retinal degenerations. An intronic change (IVS11+3G_A) was also identified in one Stargardt’s patient. None of the isocoding changes or the intron change alters a splice site. Cosegregation is pending to determine whether the observed missense sequence anomalies are pathogenic in Stargardt’s macular dystrophy.

CONCLUSIONS: We report 7 sequence changes in MFRP in patients with inherited retinal degenerations. The possible pathogenic role of these changes is under further investigation. We are proceeding with an evaluation of the remaining exons in these patients and an evaluation of all exons in additional patients with allied diseases.
Mutation Screening of RPGR in Male Patients With X-Linked or Isolated Forms of Retinitis Pigmentosa or Cone-Rod Dystrophy


PURPOSE: Mutations in the RPGR (retinitis pigmentosa GTPase regulator) gene isolated from RP3 region (Xp21.1) have been reported to be responsible for up to 70% of X-linked (XL) retinitis pigmentosa (RP) families. RPGR exon ORF15 mutations have also been shown to cause XL-atrophic macular degeneration and COD1 type XL-cone-rod dystrophy (CRD). This study is intended to determine the spectrum and frequency of RPGR mutations in our male patients with XL or isolated forms of RP or CRD.

METHODS: RPGR exons 1-14 and exon ORF15 were screened for mutations by direct PCR sequencing of samples from 5 XLRP and 11 XL-CRD families and a total of 24 male patients with isolated forms of either RP or CRD. Unlike previous reports that primarily screened isolated RP cases with severe phenotypes, we evaluated all available isolated RP males to avoid an ascertainment bias.

RESULTS: Mutations were found in 2 XLRP samples (IVS1+1G>A and ORF15+483-484delGA) and in 2 isolated RP samples (213G>A and 1404C>T). Analyses of additional samples are underway. The 213G>A (G52R) is a novel missense mutation, which would also be predicted to disrupt normal splicing and was not detected in 100 control chromosomes. The 1404C>T (R449X) mutation was found in an affected male whose mother had both normal alleles, thus representing a de novo mutation. Despite a sampling bias created by our previous efforts to recruit COD1 families, more than half of our XL-CRD families have not been mapped to COD1 region and/or lack RPGR mutations, supporting the importance of genetic heterogeneity. No RPGR mutations were detected in any isolated CRD cases.

CONCLUSIONS: Our data supports the involvement of RPGR in isolated RP. The determination of the mode of inheritance by molecular testing has major implications for genetic counseling. The comparison of phenotypes of RPGR mutation-positive patients versus negatives may provide indicators for prediction of the isolated cases at high-risk for RPGR mutations. A larger cohort will be necessary to clearly establish any correlation of phenotype with RPGR mutations. However, the phenotypes of our isolated cases with RPGR mutation are consistent with the early onset, severe RP previously reported in RP3 families.
Section 6: Ophthalmic Oncology

Arun D. Singh, A. Topham

PURPOSE: To determine the incidence of primary uveal melanoma in the United States over a 25-year period from 1973 to 1997. DESIGN: Systematic review of existing databases. PARTICIPANTS: Two thousand four hundred ninety-three patients with primary uveal melanoma (International Classification of Oncology [ICDO-2] codes C69.3 [choroid melanoma] and C69.4 [ciliary body and iris]) derived from the Surveillance, Epidemiology, and End Results (SEER) program database in the United States from 1973 to 1997.

METHODS: The significance of trend in age-adjusted incidence rate was determined using chi-square test, and 95% confidence intervals were calculated. Main Outcome Measures: The age-adjusted incidence rate.

RESULTS: There was a total of 2493 cases of uveal melanoma, representing 2.9% of all recorded cases of melanoma. Almost all cases (99.4%) were reported by the hospitals, and histopathologic confirmation was available in 81.3% of cases. The mean age-adjusted incidence of uveal melanoma in the United States was 4.3 per million (4.1–4.5; 95% confidence interval [CI]). Most cases (97.8%) occurred in the white population. There was significant variation of incidence between genders (males, 4.9 [4.6–5.2] 95% CI interval; females, 3.7 [3.5–3.9] 95% CI interval). There was no significant variation of incidence by the geographic location of the registry and over the entire period of observation (chi-square test).

CONCLUSIONS: The mean age-adjusted incidence of uveal melanoma (4.3 per million) in the United States is similar to that reported from European countries. The age-adjusted incidence rate of uveal melanoma has remained stable for the past 25 years.
Gene Expression Profiling Identifies Tumour Markers Potentially Playing a Role in Uveal Melanoma Development


PURPOSE: Microarray is a powerful tool to compare the gene expression of different tumour specimens and cell lines simultaneously and quantitatively. Intention was to get a better insight into genes that are involved in uveal melanoma tumorigenesis.

METHODS: We compared the gene expression profiles of 12 different uveal melanoma cell lines with three melanocyte cell cultures obtained from healthy donor eyes. Gene expression profiles were obtained by nylon filter arrays, containing 1176 gene spots related to cancer development. The expression levels of selected genes were validated on cell lines and primary uveal melanomas by real time RT-PCR, and were subsequently included in cluster analysis.

RESULTS: Four candidate tumour markers, Laminin Receptor 1, Endothelin 2, Von Hippel-Lindau Binding protein 1 and Cullin 2, have been selected from genes that were differentially expressed in the uveal melanoma cell lines compared to the normal uveal melanocytes.

CONCLUSIONS: In primary uveal melanomas, these four markers could discriminate between two classes of uveal melanoma, which may be indicative of a differential disease process.

Arun D. Singh, A. Topham

PURPOSE: To determine variations in 5-year relative survival rates with primary uveal melanoma in the United States over a 25-year period from 1973 to 1997.

METHODS: Systematic review of existing databases. Participants: Two thousand four hundred ninety-three patients with primary uveal melanoma, International Classification of Oncology [ICDO-2] codes C69.3 [choroid melanoma] and C69.4 [ciliary body and iris]) derived from Surveillance, Epidemiology, and End Results (SEER) program database in the United States from 1973 to 1997. The patients were stratified according to the treatment (surgery or radiotherapy). The relative 5-year survival was calculated for 2054 patients diagnosed between 1973 and 1993 by the life table method using US life expectancy tables. Main Outcome Measures: The relative 5-year survival rate.

RESULTS: Surgical treatment was performed in 1476 (72%) cases, and radiotherapy was given in 300 (15%) cases. The proportion of cases treated by radiotherapy increased progressively from 2% to 28% in 20 years. Relative 5-year survival rates ranged from 77% to 84% without a statistically significant variation.

CONCLUSIONS: Advances made in the local methods of treatment of primary uveal melanoma have not led to an improvement in survival. Systemic approaches to management of uveal melanoma are warranted.
Section 7:

Pediatric Ophthalmology
The Portal Color Sort Test – A New Touch Screen Computerized Test of Color Discrimination

Alex Melamud, E. Simpson, Elias I. Traboulsi

PURPOSE: To introduce the Portal Color Sort Test (PCST), a computer-based test of color vision, and to compare it to the Farnsworth-Munsell (FM) 100 Hue test (FMHHT) in normal subjects. The FMHHT is a widely accepted instrument for the testing of color discrimination. Its advantage over other psychophysical tests of color discrimination is its ability to distinguish trichromats into categories (superior, average, and poor). Its disadvantages include the need for special ambient illumination and the length of time it takes for its completion. The touch screen computer-based Portal Color Sort Test (PCST) has a design similar to the FM 100-Hue but consists of only 36 color plates in 4 sets of 9 plates. The test is based on the accuracy with which an individual arranges each set of 9 plates on a computer screen from one shade of one color to another shade of another color.

METHODS: 10 subjects with presumed “normal” trichromatic vision and without known eye disease have been recruited to date. Each subject underwent a series of color vision tests that included the 15 plate Ishihara test, the D-15 FM test, the FM-100 hue test and the PCST under rigorous standardized conditions and as recommended by the respective manufacturers. The PCST was administered twice; once at the beginning and once at the end of the session. Tests were recorded and scored according to the manufacturers’ instructions.

RESULTS: Of the ten trichromats tested, 3 received “Superior” scores (<20) on the FMHHT and received a score of 0 (no error) on the PCST. 7 subjects tested “Average” (score = 20-100) on the FMHHT and had error scores of 0 to 12 (average 5.57) on the PCST. On repeated administration of the PCST, all but one of these 7 subjects received a perfect score of 0. The one subject who had a score of 4 on his second attempt had the highest error score of 12 on the first attempt, and the second highest error score on the FMHHT; he probably has poorer color discrimination than the rest of subjects in the FMHHT “Average” category. The average time to complete the FMHHT was 14 minutes. The average time to complete the PCST was 3 minutes.

CONCLUSIONS: Our preliminary results indicate that the PCST allows an approximately 4 – 5 times faster testing of color discrimination than the FMHHT. Subjects who have excellent color discrimination ability on the FMHHT have perfect scores on the PCST; those with average color discrimination ability on FMHHT have error scores of 0-12 on the PCST. Additional data is currently being collected to define the range of scores in the different categories of color discrimination abilities on the PCST, including patients with known color vision defects.
Improvement in Stereoacuity is Less than Expected After Treatment of Anisometric Amblyopia in Children without Strabismus

B.I. Riemann, C.D. Riemann, Sue Crowe, Elias I. Traboulsi

PURPOSE: To determine the relationship between stereoacuity (SA) and visual acuity (VA) in children with anisometropic amblyopia (AA).

Methods: SA and VA were retrospectively analyzed before and after amblyopia treatment in 44 children with AA.

RESULTS: SA improved from 200 to 152 degrees of stereo arc (p< 0.005) and VA in amblyopic eyes improved from 0.3 ± 0.2 to 0.5 ± 0.3 (p<0.0000001) after amblyopia treatment. The improvement in SA was markedly less than expected given the improvement in VA. (p<0.005) using both internal and historic controls.

CONCLUSION: The modest improvement in SA in the setting of a larger improvement in VA suggests the existence of “stereoscopic amblyopia” as an entity which is distinct from and less responsive to treatment than amblyopia of VA in orthotropic children with AA.
Familial Horizontal Gaze Palsy: Report of a UAE Family Demonstrating Autosomal Recessive Inheritance

Elias I. Traboulsi, L.J. Wang, Q. Wang, A. Mousawi, E. Engle

PURPOSE: To report a UAE family with 5 siblings affected with horizontal gaze palsy.

METHODS: Clinical examination and surgery on four affected members of a family comprised of five affected siblings, five unaffected siblings and consanguineous parents.

RESULTS: Two brothers and two sisters (ages 4 - 17 years) had congenital bilateral esotropia and abnormal eye movements since birth. The clinical manifestations were identical in all four patients. There was no ptosis. Abduction was severely limited bilaterally. Vertical ocular movements were normal. There were convergence-like eye movements on attempted abduction in lateral gaze. Visual acuity was very good in all patients, reflecting probable alternating fixation. Forced duction testing under general anesthesia revealed very tight medial rectus muscles in all patients. None of the children had severe scoliosis. Bilateral medial rectus recession resulted in good ocular alignment in primary position of gaze without any alteration of the abnormal ocular movement pattern.

CONCLUSION: This large family appears to have the rare disorder known as recessive horizontal gaze palsy with progressive scoliosis.
A Prospective, Pilot Study of Treatment of Amblyopia in Children 10 to <18 Years Old

Diane Tucker

PURPOSE: To determine whether amblyopia can be successfully treated in older children and adolescents.

METHODS: Prospective, single group treatment trial. Sixty-six amblyopic patients aged 10 to <18 years with amblyopic eye acuity of 20/40 to 20/160 were treated with daily patching (≥2 hours a day) combined with at least 1 hour of near activities. Visual acuity was measured before and after 2 months of prescribed treatment.

RESULTS: Visual acuity improved 2 or more lines from baseline in 18 (27%) of the 66 patients (95% confidence interval, 17%–40%), and the improvement appeared similar in 10- to <14-year-olds and 14- to <18-year-olds.

CONCLUSIONS: Amblyopia treatment can improve visual acuity in older children and adolescents. A randomized controlled trial is needed to determine if there is an upper age limit for which amblyopia treatment is successful.
Section 8:

Refractive
Clinical Importance and Sensitivity of Wavefront Analysis in Normal and Highly-Aberrated Eyes

Ronald R. Krueger, Maria Regina Chalita, Samra Waheed

PURPOSE: To assess wavefront measurements in normal and highly aberrated eyes and to describe the practical application of wavefront interpretation.

METHODS: Wavefront maps from the LADARWave wavefront measurement device were evaluated in different conditions such as subclinical keratoconus, post penetrating keratoplasty, post penetrating trauma, symptomatic post LASIK patients, keratoectasia and post LASIK with flap microstriae. Higher order aberrations were correlated to clinical findings and symptoms. Pupil diameter impact in wavefront measurements was evaluated.

RESULTS: The LADARWave wavefront measurement device was able to capture lower and higher order aberrations in highly aberrated eyes with good reproducibility. It was also able to capture subtle aberrations such as the ones caused by microstriae. Pupil diameter played a paramount role in higher order aberrations evaluation, changing not just the amount but also the pattern of aberrations (dominant Zernike term).

CONCLUSIONS: The LADARWave wavefront measurement device had a large dynamic range of +6.0 to -15.0 D with an ability to capture highly aberrated eyes. Aberrations were pupil size dependent, changing their dominant Zernike term with a change in pupil radius. This device could also capture very subtle aberrations. The LADARWave device defined aberrations that correlated with visual symptoms, helping in understanding patient’s complaints.
Wavefront Analysis of Flap and Laser-induced Aberrations in a Two-Step LASIK Procedure

Ronald R. Krueger, Maria Regina Chalita, Samra Waheed

PURPOSE: To identify aberrations created by making a flap only for LASIK and by treating the LASIK refractive error with a flying-spot laser.

METHODS: Twenty-six eyes were submitted to a two-step LASIK with the Autonomus LADARVision laser. In the first step the flap was made, and 1 month later it was lifted and the laser correction was done. Aberrations were measured with the LADARWave wavefront measurement device preoperatively, after making the flap (1 day, 1 week, 1 month post flap) and after laser treatment (1 week, 3 months post laser). Two different microkeratomes were used (Moria M2 and SKBM). With SKBM all flaps were nasal, with Moria M2 the flap was randomly selected as superior or nasal.

RESULTS: Making the flap promotes changes in lower and higher order aberrations. There was a slight hyperopic shift after making the flap noted in the manifest refraction and in the wavefront refraction. The higher order aberrations also change, especially the amount of coma and coma axis. Coma axis was also correlated to hinge placement. At 1-month post flap, coma changes tend to return to its pre-operative pattern. After laser treatment, spherical aberration is the main aberration that increases.

CONCLUSION: Making the flap creates changes in the aberrations, but these changes seem to be transitory, returning close to pre-operative levels 1 month later. Laser treatment increases aberrations, especially spherical aberration, and these changes seem to be stable and permanent after 3 months.
Surface Ablation with Topical Mitomycin C for High Myopia Correction

Ronald R. Krueger, Maria Regina Chalita

PURPOSE: Evaluate refractive outcome and haze incidence of surface ablation procedures (Laser Epithelial Keratomileusis-LASEK, and Photorefractive Keratectomy-PRK) with the use of topical mitomycin 0.02% intraoperatively for high myopia correction.

METHODS: Retrospective noncomparative single surgeon case series. After laser ablation, a 8.0mm round sponge soaked with mitomycin 0.02% solution was applied on the stromal bed for 2 minutes and then removed. Corneal haze, BCVA, UCVA and manifest refraction were the parameters evaluated.

RESULTS: Of all primary treated eyes, the greatest haze grade found was 0.5. Eyes that were previously treated and had haze, when retreated with combined use of mitomycin, showed significant improvement in haze grade. No complications due to mitomycin use were reported and no reepithelization delay was noted.

CONCLUSIONS: Surface laser correction, in spite of being safe and predictable, can lead to corneal haze, myopic regression, discomfort and delayed recovery. Topical mitomycin is a safe adjunct in eyes with high correction, preventing haze occurrence and consequent regression, and making surface treatments much more reliable.
First Safety Study of Femtosecond Laser Photodisruption in Animal Lenses: Tissue Morphology and Cataractogenesis


PURPOSE: Refractive surgery is beginning to focus on presbyopia correction. Photophaco-modulation is one attempt to restore accommodation by modifying lens tissue with a laser. Safety studies in animal lenses can determine the tissue effects and potential cataractogenesis.

METHODS: Six fresh porcine lenses and 6 living rabbit eyes are irradiated with a femtosecond laser (Ti:Sapphire, 130 fsec). Several hundred thousand pulses with an energy of 1-4 μJ/pulse are applied to the lens epinucleus in one of two patterns: 3 concentric annuli or 8 radial slits. The rabbit eyes are treated according to ARVO guidelines, leaving one eye as a control. Both rabbit lenses are tested for light scatter with a low power He-Ne laser 3 months after the treatment, and then photographed and fixed for ultrastructure. Porcine eyes are fixed after lasing.

RESULTS: After the laser treatment, all lenses display bubbles which resolve with time. In the porcine eyes, the bubbles coalesce for a spacing pattern of > 9µm with pulse energy of 2 μJ. In the rabbit eyes, an energy of 1 μJ and spacing of 10µm is chosen for transcorneal delivery. Ultrastructurally, the porcine eyes demonstrate a 0.5µm electron dense border layer with adjacent normal lens architecture. After 3 months, the rabbit lenses demonstrate good transparency with only a fine optical distortion at the site of laser treatment. One rabbit, in both eyes, had cataract formation unrelated to the laser. In the other 5 rabbits, laser scanning studies reveal essentially identical values for the back vertex distance, sharpness of focus, and light scatter compared to the control.

CONCLUSION: Femtosecond laser photodisruption of the ocular lens yields discrete lesions with a border zone of ~0.5µm, and bubbles which resolve with time. In the living animal eyes, no cataract formation can be found after 3 months, and there is a similar depth of focus and value of scatter when compared to fellow eyes. These preliminary results suggest that femtosecond laser can be safely used in modifying the paracentral lens nucleus/epinucleus for presbyopia correction.
Wavefront Analysis and its Correlation with Refraction and Topography in Normal Eyes
Sai H. Chavala, Maria Regina Chalita, Samra Waheed, Meng Xu, Ronald R. Krueger

PURPOSE: To evaluate the information accessed with the LADARWave wavefront measurement device and correlate it with the clinical findings of refraction and computerized corneal topography.

METHODS: 60 eyes (30 patients) of healthy individuals being evaluated by preoperative exam; 14 male (46.66%): 16 female (53.33%); Mean age: 39.16 years (23-63 years); Exclusion criteria: keratoconus, corneal scars, irregular ocular surface, corneal dystrophies, previous ocular surgery, cataract.

Ophthalmological exam:
• manifest refraction in a dark room
• cycloplegic refraction
• computerized corneal topography (Zeiss-Humphrey, Dublin, CA)
• dilated wavefront measurement (LADARWave, Alcon, Ft Worth, TX).
• scotopic pupil size

LADARWave device
• Shack-Hartmann aberrometer
• Low order aberrations:
  • Defocus
  • Astigmatism
• Higher order aberrations:
  • Coma
  • Spherical aberrations
  • Other terms
• Match percentage

STATISTICAL ANALYSIS: Pearson’s correlation coefficient for 2 continuous variables (0: no linear correlation; ±1 perfect linear correlation)

RESULTS: Mean match percentage: 85%
Mean values:
• coma = 0.35mm (SD= 0.29)
• spherical aberrations = 0.36mm (SD= 0.31)
• other terms = 0.31mm (SD= 0.14)
• total aberration= 1.05 mm (SD= 0.47)
Association between manifest/cycloplegic refraction and wavefront refraction:

<table>
<thead>
<tr>
<th></th>
<th>M sph</th>
<th>C sph</th>
<th>M cyl</th>
<th>C cyl</th>
<th>M axis</th>
<th>C axis</th>
</tr>
</thead>
<tbody>
<tr>
<td>W sph</td>
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<td>0.98</td>
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<tr>
<td></td>
<td>(0.95,0.98)</td>
<td>(0.97,0.99)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
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<td></td>
</tr>
<tr>
<td>W cyl</td>
<td>0.47</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>(0.25,0.65)</td>
<td>(0.35,0.71)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>p=0.007</td>
<td>p=0.001</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>W axis</td>
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<td></td>
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<td>0.81</td>
<td></td>
<td></td>
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<tr>
<td></td>
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<td>(0.20,0.64)</td>
<td>(0.68,0.89)</td>
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<tr>
<td></td>
<td></td>
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<td>p=0.021</td>
<td>p&lt;0.001</td>
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Association between manifest/cycloplegic refraction with wavefront refraction, considering match subgroups:

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<th>C sph</th>
<th>M cyl</th>
<th>C cyl</th>
<th>M axis</th>
<th>C axis</th>
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</thead>
<tbody>
<tr>
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<td>≥85%</td>
<td>(0.96,0.99)</td>
<td>(0.98,0.99)</td>
<td>(-0.05,0.52)</td>
<td>(0.28,0.72)</td>
<td>(0.20,0.70)</td>
<td>(0.67,0.91)</td>
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<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p=0.27</td>
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<tr>
<td>W cyl</td>
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<td>0.35</td>
<td>0.76</td>
<td>0.67</td>
<td>0.19</td>
<td>0.59</td>
</tr>
<tr>
<td>&lt;85%</td>
<td>(-0.38,0.55)</td>
<td>(-0.18,0.72)</td>
<td>(0.45,0.91)</td>
<td>(0.26,0.88)</td>
<td>(-0.36,0.64)</td>
<td>(0.02,0.87)</td>
</tr>
<tr>
<td></td>
<td>p=0.79</td>
<td>p=0.43</td>
<td>p=0.027</td>
<td>p=0.10</td>
<td>p=0.72</td>
<td>p=0.41</td>
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Tendency toward greater wavefront cylinder:
- 63.3% of eyes (manifest cylinder); - 65% of eyes (cycloplegic cylinder)

Association of refractive components with total aberrations:

<table>
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<tr>
<th></th>
<th>M sph</th>
<th>C sph</th>
<th>W sph</th>
<th>M cyl</th>
<th>C cyl</th>
<th>W cyl</th>
<th>M axis</th>
<th>C axis</th>
<th>W axis</th>
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<tr>
<td>Total</td>
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<td>0.22</td>
<td>0.18</td>
<td>0.05</td>
<td>0.10</td>
<td>0.22</td>
<td>0.005</td>
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<tr>
<td>Aber</td>
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<td>p=0.33</td>
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<td>p=0.23</td>
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Association of refractive components with total aberrations, considering match subgroups:

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<th>W sph</th>
<th>M cyl</th>
<th>C cyl</th>
<th>W cyl</th>
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<tr>
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<td>0.17</td>
<td>0.30</td>
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<td>0.16</td>
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<td>≥85%</td>
<td>(0.48,0.48)</td>
<td>(0.48,0.48)</td>
<td>(0.21,0.21)</td>
<td>(0.14,0.43)</td>
<td>(0.55,0.55)</td>
<td>(0.19,0.19)</td>
<td>(0.94,0.94)</td>
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<td></td>
<td>p=0.48</td>
<td>p=0.44</td>
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<td>p=0.43</td>
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<tr>
<td>Match</td>
<td>0.55</td>
<td>0.43</td>
<td>0.007</td>
<td>0.03</td>
<td>0.04</td>
<td>0.30</td>
<td>0.62</td>
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<tr>
<td>&lt;85%</td>
<td>(0.16,0.34)</td>
<td>(0.99,0.94)</td>
<td>(0.94,0.94)</td>
<td>(0.94,0.94)</td>
<td>(0.51,0.19)</td>
<td>(0.29,0.29)</td>
<td>(0.89,0.89)</td>
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<tr>
<td></td>
<td>p=0.16</td>
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<td>p=0.99</td>
<td>p=0.94</td>
<td>p=0.51</td>
<td>p=0.19</td>
<td>p=0.29</td>
<td>p=0.89</td>
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Association of refractive components with topography:

<table>
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<th>M cyl</th>
<th>C cyl</th>
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<th>M axis</th>
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<th>W axis</th>
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<tbody>
<tr>
<td>Topo</td>
<td>0.03</td>
<td>0.10</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astig</td>
<td>(-0.23,0.28)</td>
<td>(-0.16,0.35)</td>
<td>(-0.13,0.37)</td>
<td>p=0.89</td>
<td>p=0.60</td>
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<tr>
<td>Topo</td>
<td>0.39</td>
<td>0.26</td>
<td>0.16</td>
<td></td>
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</tr>
<tr>
<td>Axis</td>
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<td>(-0.26,0.26)</td>
<td>p=0.046</td>
<td>p=0.24</td>
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Association of higher order aberrations with scotopic pupil size:

<table>
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<tr>
<th>Coma</th>
<th>Sph Aber</th>
<th>Other Terms</th>
<th>Total Aber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pupil Diameter</td>
<td>0.16</td>
<td>0.29</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>(-0.10,0.40)</td>
<td>(0.04,0.51)</td>
<td>(-0.24,0.27)</td>
</tr>
<tr>
<td></td>
<td>p=0.40</td>
<td>p=0.052</td>
<td>p=0.91</td>
</tr>
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</table>

CONCLUSIONS:
In the majority of eyes, higher order aberrations were less than: 0.64 µm of coma (mean +SD); 0.67 µm of spherical aberration (mean +SD); 0.45 µm of other terms (mean +SD)

Refration
- Wavefront sphere highly correlated to manifest and cycloplegic sphere
- Wavefront cylinder tends to be higher than manifest and cycloplegic cylinder
- High match subgroup have higher correlation with refraction data

Topography
- Topographic axis correlated to manifest axis

Scotopic Pupil size
- The bigger the scotopic pupil, the more spherical aberration

The LADARWave device is a valuable diagnostic tool in measuring refractive errors and ocular aberrations in normal eyes
It is helpful in preoperative surgical planning.
Wavefront Analysis in Normal Refractive Surgery Candidates
Marcelo V. Netto, Renato Ambrosio, Jr., Tung Shen, Steven E. Wilson

PURPOSE: To quantify the higher order aberrations (HOA) of refractive surgery candidates and compare the wavefront refraction with manifest and cycloplegic refraction.

METHODS: Results of 226 consecutive patients (418 eyes) were analyzed with the WaveScan (Visx, Santa Clara, CA). Only patients with normal eyes that had not had previous surgery were included.

RESULTS: The mean spherical equivalent (SE) was -3.40 diopters (D) with a standard deviation (SD) of ±3.14 D (range -10.72 to +5.41 D). The most significant higher order aberrations were detected under maximum pupil size of 6 mm (coma 0.14 ± 0.08µm; trefoil 0.10 ± 0.07µm; spherical equivalent 0.09 ± 0.07µm). There was no statistically significant correlation between high order aberrations and gender (p=0.78), age (p>0.63) or spherical equivalent (p>0.59). The mean differences in SE, sphere and cylinder between WaveScan measurements and manifest refraction were 0.36 D ± 0.41 D, 0.40 ± 0.44 D and 0.28 ± 0.32 D, respectively. The correlation was significantly less for high refractive errors.

CONCLUSION: Wavefront analysis proved to be a valuable tool for measuring pre-operative higher order aberrations and refractive error. This study also provides reference values for normal refractive surgery candidates.
Corneal Surface Smoothing after Refractive Surgery
David Huang, Maolong Tang, Raj Shekhar

PURPOSE: To construct a quantitative model of corneal surface smoothing after laser ablation for refractive correction. Design: Experimental study, interventional case series, and meta-analysis of literature.

METHODS: A theory of epithelial smoothing in response to corneal contour change is derived from differential equations that describe epithelial migration, growth, and loss. Computer simulations calculate the effects on postoperative epithelial thickness, topography, refraction, and spherical aberration. Model parameter is matched with laser in-situ keratomileusis (LASIK) outcome in literature and in a retrospective study of primary spherical myopic (77 eyes), and hyperopic (19 eyes) corrections. Surgically-induce refractive change was the main outcome measure.

RESULTS: Simulated epithelial remodeling after myopic ablation produces central epithelial thickening, reduction in achieved correction, and induction of oblate spherical aberration. Simulation of hyperopic ablation shows peripheral epithelial thickening, a larger reduction in correction, and induction of prolate spherical aberration. Simulation using a minus cylinder laser ablation pattern shows decreased astigmatism correction and increased hyperopic shift. In the LASIK series, linear regression of achieved correction v. ablation setting in hyperopic, and minus cylinder corrections shows slopes of 0.97, 0.71, and 0.74, respectively. These clinical results match model predictions when the smoothing constant is set at 0.32, 0.63, and 0.55 mm, respectively.

CONCLUSION: Epithelial thickness modulations after ablation can be modeled mathematically to explain clinically observed regression and induction of aberration. The cornea appears to smooth over ablated features smaller than approximately 0.5 mm. The model provides an approach for designing ablation patterns that pre-compensate for the smoothing to improve final outcome.
Correlation of Pupil Sizes Measured with a Mesopic Infrared Pupillometer and a Photopic Topographer

L.M. Periman, Renato Ambrosio, Jr, D.A. Harrison, Steven E. Wilson

PURPOSE: To assess pupil size measurements obtained under scotopic and mesopic conditions with the Procyon pupillometer and under photopic conditions with the Humphrey videokeratographer.

METHODS: The pupil sizes of 96 candidates for refractive surgery were measured with the Procyon pupillometer PS2000 SA (Keeler, Broomall, PA) and the Humphrey Atlas 992 corneal topographer (version A9, San Leandro, CA). Anisocoria and pupillary unrest were analyzed according to gender (2 groups: of 51 females and 45 males), age (5 groups: 20-30 yo, 31-40yo, 41-50yo, 51-60yo and greater than 60yo) and level of refraction (5 groups: > -6.00 diopters spherical equivalent (SE), -6.00 to -3.00 diopters SE, -3.00 to 0 diopters SE, 0 to +2.50 diopters SE, and + 2.50 to +5.00 diopters SE).

RESULTS: The median value of pupil diameter measured with the Procyon pupillometer at the scotopic (0.04 lux), mesopic-low (0.4 lux) and mesopic-high (4 lux) levels of illumination were 6.54 mm ± 0.88 (SD) mm; 5.62 ± 0.95 mm, and 4.09 ± 0.76 mm, respectively. The median pupil size with the Humphrey topographer was 3.65 ± 0.62 mm. Pupillary unrest was highest at the mesopic-high level of illumination, with the median value being 0.31 ± 0.34 mm. Median pupil size measured with both instruments at all light levels dropped significantly after the fifth decade of life (P<0.05, ANOVA).

CONCLUSIONS: The Procyon pupillometer and Humphrey videokeratograph revealed an inverse correlation between the pupil size and the age, but no relationship with sex or level of refraction. The Procyon pupillometer provides an objective method for measuring pupil size at controlled light levels with a permanent printed record.
Section 9:

Retina
Subretinal Fibrinolysis of Submacular Hemorrhage without Surgical Drainage
Hilel Lewis, Michelle Young, Jonathan E. Sears, Peter K. Kaiser, Leonid Lerner

PURPOSE: Report our experience with subretinal fibrinolysis of submacular hemorrhage with tissue plasminogen activator followed by fluid air exchange and without surgical drainage.

METHODS: Twenty-one eyes of 21 patients with thick submacular hemorrhage were treated surgically. The mean follow-up was 10 months.

RESULTS: Sixteen eyes (76%) had improved visual acuity after surgery with 8 eyes (38%) maintaining this improvement at the last follow-up. Patients with decreased final visual acuity had either occult choroidal neovascularization or had progression of geographic atrophy.

CONCLUSION: Subretinal fibrinolysis of submacular hemorrhage without surgical drainage can improve vision in some patients.
Pneumatic Displacement of Subretinal Hemorrhage Damages the Retinal Photoreceptors

Hilel Lewis, Hirokazu Sakaguchi

PURPOSE: To determine if displacement of subretinal hemorrhage by intravitreal gas injection causes damage to retinal photoreceptors overlaying the blood clot.

METHODS: Autologous blood was injected into the subretinal space of 30 rabbit eyes transclerally. Eighteen eyes were treated with gas injection. Subretinal tissue plasminogen activator (t-PA) injection followed by gas injection was performed on five eyes. An additional five eyes had subretinal blood injection only and were used as controls. Two days after the gas injection, the eyes were enucleated and histopathologic analysis was performed.

RESULTS: In the eyes injected with gas, there was absence of photoreceptors in the area where the blood clot had been present. In the eyes treated with t-PA followed by gas injection and in the control eyes, the photoreceptors were intact.

CONCLUSIONS: Displacement of subretinal hemorrhage by intravitreal gas causes damage to retinal photoreceptors attached to the subretinal clot. Due to the irreversible nature of photoreceptor loss, we do not recommend pneumatic displacement of submacular hemorrhage.
Clinicopathologic Study After Submacular Removal of Choroidal Neovascular Membranes Treated with Verteporfin Ocular Photodynamic Therapy


PURPOSE: To report the clinicopathologic findings after submacular removal of choroidal neovascular membranes (CNV) treated with verteporfin ocular photodynamic therapy.

METHODS: Interventional case series; Retrospective review of eight eyes of eight patients who underwent submacular surgery for CNV after having previously received verteporfin ocular photodynamic therapy for presumed ocular histoplasmosis (one patient), age-related macular degeneration ([AMD] three patients) pathologic myopia (two patients), punctate inner choroiditis (one patient), and idiopathic CNV (one patient). All cases had undergone ocular photodynamic therapy with verteporfin using standard protocols. Six of eight patients suffered a submacular hemorrhage after ocular photodynamic therapy, and two of eight patients refused further ocular photodynamic therapy. All patients subsequently had submacular surgery with removal of the CNV. One membrane was routinely processed, sectioned, and stained with hematoxylin and eosin. Five membranes were stained with toluidine blue for light microscopic examination. Semithin (1.0 microm) sections were cut and stained with uranyl acetate-lead citrate for transmission electron microscopy.

RESULTS: Choroidal neovascular membranes were removed at 3 days (presumed ocular histoplasmosis), 29 days (punctate inner choroiditis), 63 days (AMD, pathologic myopia), 66 days (AMD), 107 days (pathologic myopia), 116 days (AMD), and 152 days (idiopathic) after verteporfin ocular photodynamic therapy. Histopathologic and ultrastructural examination showed areas of vascular occlusion at 3 days that were not seen at later time points. All specimens had patent CNV. There were signs of vascular damage with extravasated erythrocytes and fibrin, pigment clumping in cells, and inflammatory cells in all but the 3-day specimen.

CONCLUSIONS: This case series presents data only from patients who refused repeat treatment with ocular photodynamic therapy or who developed submacular hemorrhage after initial photodynamic therapy. Histopathologic evaluation of CNV 3 days after verteporfin ocular photodynamic therapy showed partial vascular occlusion that was not present in later specimens. These later specimens demonstrated evidence of vascular damage. Verteporfin ocular photodynamic therapy does not appear to lead to permanent and complete occlusion of the CNV. Thus, treatments that lead to permanent closure of CNV without damage to the retinal pigment epithelium and sensory retina are still needed. Copyright 2003 by Elsevier Science Inc.
Peripheral Retinal Degenerations and the Risk of Retinal Detachment

Hilel Lewis

 PURPOSE: To review the degenerative diseases of the peripheral retina in relationship with the risk to develop a rhegmatogenous retinal detachment and to present recommendations for use in eyes at increased risk of developing a retinal detachment.

 METHOD: Focused literature review and author’s clinical experience.

 RESULTS: Retinal degenerations are common lesions involving the peripheral retina and most of them are clinically insignificant. Lattice degeneration, degenerative retinoschisis, cystic retinal tufts, and very rarely, zonular traction tufts, can result in a rhegmatogenous retinal detachment. Therefore, these lesions have been considered for prophylactic therapy; however, adequate studies have not been performed to date.

 CONCLUSIONS: Well-designed, prospective, randomized clinical studies are necessary to determine the benefit-risk ratio of prophylactic treatment. In the meantime, the evidence available suggests that most of the peripheral retinal degenerations should not be treated except in rare, high-risk situations.
Vitrectomy for Diabetic Macular Edema and Detachment Associated with Posterior Hyaloidal Traction

Hilel Lewis, Jonathan E. Sears, Peter K. Kaiser

PURPOSE: To evaluate vitrectomy with removal of the posterior hyaloid for patients with diabetic macular edema and detachment associated with posterior hyaloidal traction.

METHODS: Twelve eyes of 12 patients with diabetic macular edema and detachment confirmed by optical coherence tomography were surgically treated.

RESULTS: There was macular reattachment in all 12 eyes and resolution of macular edema in 8 eyes (67%). Final visual acuity after a minimum follow-up of 6 months demonstrated improvement in 9 eyes (75%).

CONCLUSION: Vitreous surgery can improve the visual prognosis of patients with diabetic macular edema and detachment associated with posterior hyaloidal traction.
Choroidal Neovascularization in the Rat Induced by Episcleral Implantation of a VEGF Pellet

Quteba Ebrahem, Bela Anand-Apte

PURPOSE: To determine the effects of episcleral implantation of a slow release pellet containing VEGF.

METHODS: Hydroxylethylmethacrylate (hydron) pellets containing VEGF for sustained release were implanted into the episclera of Long Evans or Spraque-Dawley rats. Contralateral eyes were implanted with pellets containing buffer and served as controls. The induction of CNV was evaluated at four days, one and two weeks post implantation, using fluorescein angiography, India ink perfusion flat mounts, serial sections, light microscopy and immunohistochemistry.

RESULTS: Fluorescein angiography showed an increased permeability and leakage of affected vessels located beneath the pellet implant. Using India ink perfusion of albino rats and flat mount preparations of choroid, neovascularization was seen at the quadrant where the pellet was implanted. Retinal detachment with subretinal hemorrhage was also observed. Histology showed points of compromised Bruch’s membrane with choroidal vascular in-growth.

CONCLUSION: This model provides a relatively easy approach to produce an acute CNV using VEGF delivered episclerally, thereby avoiding the complications of an intraocular invasion procedures. Systemic and oral antiangiogenic agents can potentially be tested using this model.
Proteomic Approach: Identification of Visual Cycle Protein-Protein Interactions
Sanjoy K. Bhattacharya, Zhiping Wu, Karen A. West, Z. Jin, M. Nawrot, J.C. Saari and John W. Crabb

METHODS: Proteomic approach was used to identify the visual cycle protein interactions and to demonstrate a functional interaction between cellular retinaldehyde binding protein (CRALBP) and 11-cis-retinol-dehydrogenase (RDH5). The visual cycle is the enzymatic pathway by which all-trans-retinal from photoreceptor bleaching is isomerized to 11-cis-retinal in the retinal pigment epithelium (RPE) for visual pigment regeneration. Protein-protein interactions were sought in bovine retinal pigment epithelial (RPE) microsomes by reciprocal immunoprecipitations and other affinity isolation methods. Proteins were identified by Western analyses, MALDI-TOF MS and LC MS/MS (Qtof2).

RESULTS: Kinetic parameters for RDH5 catalyzed reactions with retinoid substrates were determined in the presence and absence of recombinant purified CRALBP. Wildtype holoCRALBP enhances the affinity of recombinant purified RDH5 for 9-cis- and 11-cis-retinoids by 2-3 fold over free retinoid. Mutant M225K rCRALBP, which lacks ligand binding capability, has no effect on RDH5 enzyme kinetics. CRALBP, RDH5 and RPE 65, PRBP, CRBP and RGR opsin co-precipitate from RPE microsomes with anti-CRALBP antibodies. Similar results were obtained with anti-RDH5, anti-CRBP and anti-RPE65 antibodies.

CONCLUSIONS: These results indicate a stable, functional interaction among these proteins and a functional interaction between CRALBP and RDH5. Visual cycle proteins CRALBP, RDH5, PRBP, CRBP, RPE65 and RGR opsin appear to interact in a protein complex. Further proteomic and affinity isolation approaches are directed toward identifying other possible RPE components of a visual cycle protein complex.
Identification of the CRALBP Ligand Binding Pocket by Photoaffinity Labeling

Zhiping Wu, Sanjoy K. Bhattacharya, Koji Nakanishi, John W. Crabb

PURPOSE: To identify the critical residues in CRALBP (cellular retinaldehyde-binding protein) ligand-binding pocket. CRALBP serves as an 11-cis-retinol acceptor and as a modulator of 11-cis-retinol dehydrogenase in the mammalian rod visual cycle. Mutations in the CRALBP gene cause progressive retinal degenerations that lead to blindness. To better understand the molecular interactions between CRALBP and its ligand, photoaffinity labeling with retinoid analogue has been pursued and labeled sites identified by mass spectrometry.

METHODS: Purified human recombinant CRALBP was labeled in the dark with 3-diazo-4-keto-11-cis-retinal (retinoid analogue). Covalent incorporation of this ligand was evaluated following photolysis with UV-light (254 nm) at –196˚C for 5s to 20 min. Protein bound retinal was reduced to retinol with NaB'3H4, radiolabeling the ligand incorporation sites. Labeled rCRALBP was denatured in 8 M urea, alkylated with iodoacetamide, digested with trypsin and peptides fractionated by RP-HPLC. Radioactive HPLC fractions were identified by scintillation counting and peptide sites of incorporation identified by MALDI-TOF MS and LC MS/MS. Short (5-40s) irradiation times yielded relatively constant incorporation levels (~1%) therefore 5s photolysis times were chosen to minimize nonspecific protein modifications. MALDI TOF MS and LC MS/MS analyses of tryptic peptides from photolabeled rCRALBP have accounted for 100% of the rCRALBP sequence.

RESULTS: Eight photoaffinity modified residues were identified, all with variable mass additions. This variability may be due to free radical migration throughout the conjugated double bonds of the retinoid analogue. Four of the photoaffinity modified sites are in authentic retinoid binding pocket residues based upon previous studies. The other four modified residues may represent newly identified CRALBP ligand binding pocket components. The functional significance of these residues is under investigation.

CONCLUSIONS: Eight amino acid residues underwent modification with photoaffinity label and were identified as part of CRALBP ligand-binding pocket.
Identification of Retinal Proteins that are Nitrated in Elevated Glucose Concentration

Y.Du, M.Miyagi, Karen West, John W. Crabb, T.S. Kern

PURPOSE: Inhibition of diabetic retinopathy in animals by aminoguanidine is accompanied by a reduction in retinal protein nitration. We sought the identity of retinal proteins that are nitrated in diabetes as an approach to better understanding the pathogenic mechanisms of this retinopathy.

METHODS: In vivo studies utilized retinas collected from STZ-diabetic rats (2 month duration), and in vitro studies used a transformed Muller cell line (rMC-1) incubated in normal (5mM) and high (25 mM) glucose. Retinas or cells were homogenized and subjected to 2D Western analysis using an anti-nitrotyrosine antibody. Immunoreactive spots were excised from the 2D gel, digested in situ with trypsin and analyzed by MALDI-TOF MS and/or LC MS/MS.

RESULTS: Nitrotyrosine immunoreactivity in Western blots of retinas from diabetic rats was greater than that from nondiabetic rats or from diabetic rats treated with aminoguanidine. Likewise, rMC-1 cells incubated in 25 mM glucose exhibited greater nitrotyrosine immunoreactivity in Western blots than cells incubated in 5 mM glucose or in 25 mM glucose + aminoguanidine. The same nitrotyrosine immunoreactive proteins were identified from retinal homogenates and cultured rMC-1 cells, and include alpha enolase, glyceraldehyde 3-phosphate dehydrogenase, voltage-dependent anion-selective channel protein, aldolase, triosephosphate isomerase. Many of the identified nitrotyrosine immunoreactive proteins appear to be glycolytic enzymes. Alpha enolase exhibited the most dramatic change in apparent nitrotyrosine content.

CONCLUSIONS: Hyperglycemia appears to increase nitration of glycolytic enzymes in retina and retinal cells. NO or nitration can alter enzyme activities of proteins. The present findings justify further consideration of nitration and nitric oxide as possible mediators in the mechanisms of diabetic retinopathy.
CRALBP Topological Analyses by Mass Spectrometry

John W. Crabb, A.Hasan, Z.Wu

PURPOSE: Cellular retinaldehyde binding protein (CRALBP) interacts with other proteins as well as with ligand in its role as an acceptor of 11-cis-retinol and substrate carrier in the rod visual cycle. CRALBP topological analyses have been pursued as an approach to identifying functional domains and better understanding visual cycle mechanisms.

METHODS: Human recombinant CRALBP with a 23 residue His-tag N-terminal fusion sequence was produced in E. coli and purified to apparent homogeneity. Amide hydrogen-deuterium exchange (H-D exchange) and mass spectrometric analyses of pepsin digests of apo- and holo-rCRALBP were used to identifying solvent exposed regions that may interact with other proteins and buried regions that may bind 11-cis-retinoid.

RESULTS: Significant localized differences in deuterium incorporation were found between apo- and holo-rCRALBP. Ligand dependent conformational changes were observed in rCRALBP residues 4-22, 80-94 and 282-316 which are more solvent exposed in the holo-protein and in residues 198-212 and 224-243 which are less solvent exposed in the holo-protein. Other regions exhibited less than 5% difference in deuterium incorporation between the apo- and holo-proteins.

CONCLUSIONS: Binding of 11-cis-retinal induces conformational changes in rCRALBP detectable by H/D exchange mass spectrometry. Separate analyses have confirmed retinoid binding pocket components within buried holo-rCRALBP residues 198-212 and 224-243. The present results provide useful hints regarding structural domains in CRALBP available for functional interactions.
Proteomic Analysis of Vitreous in the Presence of Diabetic Macular Edema

M.Ouchi, M.Kamei, Karen A.West, T.Yasuhara, M.Tei, H.Komori, T.Yamamoto, S.Kinoshita, John W. Crabb

PURPOSE: Even patients with posterior vitreous detachment show improvement in their diabetic macular edema (DME) after vitreous surgery. This may be attributable to the removal of chemical mediators present in the posterior vitreous cortex. Earlier studies of DME-related proteins have focused on single proteins such as interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF). We are pursuing a global approach to identifying DME-related proteins using 2D electrophoresis and mass-spectrometry (MS).

METHODS: We divided 44 patients who had undergone vitreous surgery into 4 groups; those with a macular hole/epiretinal membrane (MH/ERM) (n=11, 11 eyes); patients with non-DME pre-proliferative diabetic retinopathy (PPDR) (n=4, 4 eyes); those with DME (n=14, 16 eyes); and patients with proliferative diabetic retinopathy (PDR) (n=15, 15 eyes). Vitreous (~300 ul) were collected from the pre-macular vitreous body, total protein determined by the blood-fold method, and ~15 ug samples subjected to 2D electrophoresis and stained with SYPRO-Ruby. Proteins unique to DME vitreous were determined by comparison of gel patterns using image analysis software, excised from the gel, digested in situ with trypsin and identified by LC MS/MS sequence analysis.

RESULTS: Compared with the PPDR group, the DME group exhibited many 2D gel spots with significantly greater staining intensities. Furthermore, three of the prominently demarcated spots from the DME group were identified as pigment epithelium derived factor (PEDF), apoliprotein A-4 (ApoA-4), and thyroid hormone receptor-interacting protein-11 (Trip-11).

CONCLUSIONS: These findings suggest that PEDF, Apo-4 and Trip-11 may play a role in the pathogenesis of DME. The cytokine PEDF is involved in several retinal diseases, and high levels of the ApoA-4 are found in sera from diabetics and patients with renal failure. Further study of the possible relationships between these endogenous factors and DME is warranted.
CRALBP Interacts with a PDZ-domain Protein in Extracts of RPE

J.C. Saari, M.Nawrot, Karen A. West, John W. Crabb

PURPOSE: Analysis of the phenotype of cellular retinaldehyde-binding protein (CRALBP) knockout mice led us to conclude that the visual cycle was delayed at the isomerase step because of the lack of an efficient acceptor for 11-cis-retinol. Earlier studies in vitro demonstrated that 11-cis-retinol bound to CRALBP was a good substrate for a cis-specific dehydrogenase of RPE. Thus, it is likely that apo-CRALBP accepts 11-cis-retinol from an isomerase and facilitates its oxidation to 11-cis-retinal. In order to understand the mechanism of release of 11-cis-retinal from CRALBP and from RPE, we sought proteins that interact with CRALBP in RPE.

METHODS: Interacting proteins were detected with an overlay assay. RPE microsomes were subjected to 1D or 2D SDS PAGE and blotted to a PVDF membrane, which was blocked, incubated with CRALBP, washed and probed with anti-CRALBP. Proteins were excised from the gel, digested in situ with trypsin and identified by LC MS/MS sequence analysis.

RESULTS: CRALBP bound to a protein with an apparent molecular weight of 54 kDa in 1D gels. Other proteins substituted for CRALBP in the assay did not bind, suggesting a specific interaction. The protein was resolved into several components on 2D gels and each was identified by sequence analysis as ERM-binding phosphoprotein 50 (EBP50), also known as sodium/hydrogen exchanger-3 regulatory factor (NHERF-1).

CONCLUSIONS: ERM (ezrin, radixin, moesin) proteins link plasma membrane proteins with the actin cytoskeleton. EBP50 is a phosphoprotein that binds to ERM proteins through its C-terminal domain. It is known to colocalize with ezrin in apical RPE. It also interacts through its two PDZ-domains with a number of other proteins, including sodium/hydrogen exchanger-3. The functional significance of the affinity of EBP50 for CRALBP is not known, but it may involve recruitment of the binding protein to an RPE apical protein complex involved in releasing or processing 11-cis-retinal.
Possible TULP1 Protein Interactions
Q.Xi, Karen A. West, John W. Crabb, Stephanie A. Hagstrom

PURPOSE: TULP1, a member of a family of four proteins with unknown function designated tubby-like proteins or TULPs, is expressed specifically in the inner segments and connecting cilium of photoreceptor cells. Mutations in TULP1 are associated with autosomal recessive retinitis pigmentosa and Tulp1 knockout mice develop an early-onset, progressive photoreceptor degeneration involving both rods and cones. To explore the physiologic function of TULP1, we are pursuing the identification of interacting proteins.

METHODS: Immunoprecipitation experiments were performed with bovine retinal homogenates and a polyclonal anti-TULP1 antibody. Immunoprecipitation products were separated by SDS-PAGE, protein bands excised, digested in situ with trypsin and identified by LC MS/MS.

RESULTS: The following proteins were immunoprecipitated: Microtubule Associated Protein 1B, Clathrin Heavy Chain, Interphotoreceptor Retinoid Binding Protein, Dynamin-1, Rab Geryl Geryl Transferase, Dynein Intermediate Chain, Tubulin and Actin. In vitro pull-down experiments and in vivo co-immunoprecipitation experiments are in progress to further evaluate these possible TULP1 interactions.

CONCLUSIONS: Several of the identified proteins are involved in various aspects of vesicle transport or protein movement. TULP1 may function in intracellular trafficking of proteins synthesized in the inner segment to the outer segment of photoreceptor cells. These results provide a first step toward defining the mechanism underlying photoreceptor degeneration caused by mutations in TULP1.
Light Induced Protein Modifications and Lipid Oxidation Products in Rat Retina


PURPOSE: To better understand the mechanisms of oxidative damage in retinal pathology, we have sought the identity of lipid oxidation products and protein adducts in rat retina after in vivo exposure to damaging light.

METHODS: Albino rats maintained in a dark environment for 2 months were exposed to intense green light (1500 lux) for 1 or 4 hours and sacrificed immediately following light treatment. Retinas were isolated and immediately protected with antioxidants. Lipids were extracted with chloroform/methanol and analyzed by LC MS. Proteins were extracted with SDS-PAGE sample buffer and analyzed by Western blotting.

RESULTS: Lipid oxidation products in rat retina from docosahexaenoyl phosphatidylcholine (DHA-PC), arachidonoyl (AA)-PC, and linoleyl (LA)-PC were more abundant after 4h of light exposure than after 1h or no light. Anti-carboxyethylpyrrole, anti-argpyrimidine and anti-nitrotyrosine immunoreactivities were significantly greater after 4h light exposure compared with control animals maintained in the dark. Anti-opsin immunoreactivity was also significantly greater after light treatment.

CONCLUSIONS: Current results are consistent with our recent observation that light modulates protein nitration in rat retina (2002 Mol. & Cell. Proteomics 1, 293). Intense light also generates lipid oxidation products in rat retina in vivo that result in oxidative protein modifications such as carboxyethylpyrrole from DHA containing lipids. Argpyrimidine, derived from methylglyoxal, appears to be another protein modification induced by light. The apparent increase in opsin after light may be due to modifications that increase the solubility and extractability of this integral membrane protein. These findings justify further consideration of lipid oxidation products and protein modifications as mediators in the light-induced biochemical sequel leading to photoreceptor cell death.
Analysis of Phosphorylation of Connexin 36 in Bovine Retina

A. Sitaramayya, John W. Crabb, A. Margulis, D.F. Matesic, V. Singh, S. Pulukuri

PURPOSE: Gap junction-mediated intercellular communication between specific retinal neurons is known to be regulated by cyclic nucleotide-dependent protein kinases. Connexin 36 is a major gap junction protein in retina. We attempted here to demonstrate phosphorylation of connexin 36 by kinases responsive to cyclic nucleotides and calcium.

METHODS: Retinal homogenate was phosphorylated using 32P-ATP in the presence of cyclic AMP, cyclic GMP or calcium. Proteins of the membrane fraction and membranes enriched in gap junctions were resolved by electrophoresis, and phosphoproteins were detected by autoradiography. Phosphorylated membrane proteins were also dissolved in detergent and connexin 36 was immunoprecipitated with an anti-connexin 36 antibody. Immunoprecipitated proteins were separated by 1- or 2-D electrophoresis and phosphorylated proteins were detected by autoradiography. Proteins of interest were excised from 1- or 2-D gels, digested in situ with trypsin and identified by capillary LC MS/MS.

RESULTS: Specific retinal membrane proteins were phosphorylated in response to activation by cyclic AMP, cyclic GMP or calcium. Of them, a protein of about 36 kDa was phosphorylated only in incubations with calcium. However, immunoprecipitated connexin 36 was found not to be phosphorylated. The immunoprecipitate did contain other phosphorylated proteins, and the most prominent of them, a 58 kDa protein, was identified as beta tubulin. Alpha tubulin and trypsin inhibitor were also detected in the immunoprecipitate.

CONCLUSIONS: These results suggest that connexin 36 may not be directly phosphorylated in response to changes in cyclic nucleotides or calcium. Proteins associated with connexin 36, though not necessarily the ones we identified so far, might be targets of phosphorylation and could be involved in the regulation of gap junction intercellular communication.
Cloning and Characterization of Mouse SPACRCAN

Qiuyun Chen, Kazutoshi Nishiyama, JungWha Lee, Mary E. Rayborn, Karen G. Shadrach, Joe G. Hollyfield

PURPOSE: The interphotoreceptor matrix (IPM) has been implicated in providing supportive micro-environments for photoreceptors. A number of specific molecules are novel to the IPM, but their roles have not been clearly established. Detailed analysis of specific IPM molecules should yield important information as to their function. As a first step towards this goal, we have cloned and initiated characterization of SPACRCAN in mouse retina.

METHODS: PCR amplification of a mouse retina cDNA library and 5’RACE were used to clone the cDNA. The cDNA sequence was used as a template for a Blast search for its corresponding genomic sequence. Northern blot analysis was used to study its tissue specific expression pattern. RT-PCR and immunocytochemistry were used to study its developmental expression pattern in retina.

RESULTS: Like its homolog in human and rat, mouse SPACRCAN has a signal peptide at the N-terminal, a large central mucin domain with numerous N-link and O-link glycosylation sites, two GAG attachment sites, four HA-binding motifs, two EGF-like domains, and a hydrophobic stretch of a 24 amino acid near the C-terminal. Comparison of the genomic structure between mouse and human SPACRCAN showed significant structure conservation. Analysis of the promoter regulatory region revealed several important regulatory elements, including Ret-1/PCE-1, six copies of PIRE, Ret-4, three copies of AP-1, CRE, and five copies of GATA3. Northern blot analysis showed that SPACRCAN mRNA is specifically expressed in retina and pineal gland. Like the rat, SPACRCAN mRNA in mouse was detectable as early as embryonic day 15. However, the earliest stage that SPACRCAN immunoreactivity could be detected was at postnatal day 8, when photoreceptor outer segments are in the initial process of elongation.

CONCLUSION: The presence of numerous unique regulatory elements in the promoter region may be involved in determining SPACRCAN’s developmental and tissue specific expression pattern. Early expression of SPACRCAN and its characteristic molecular features suggest that SPACRCAN may be important in early development of photoreceptor outer segments.
SPACRCAN in the Interphotoreceptor Matrix of the Mouse Retina: Molecular, Developmental and Promoter Analysis


PURPOSE: SPACRCAN is a novel proteoglycan present in the interphotoreceptor matrix (IPM) of the rat and human retina that resists aqueous extraction through its binding to hyaluronan. The purpose of this study was: to clone mouse Spacrcan; to characterize the promoter elements; to define the deduced amino acid sequence; to establish the time of Spacrcan expression during retinal development; and to determine the time of appearance and distribution of SPACRCAN protein.

METHODS: Spacrcan cDNA clone was obtained through PCR amplification of a mouse retina cDNA library, and RT-PCR amplification and 5’RACE of mouse retina RNA. The deduced polypeptide sequence of mouse SPACRCAN contains a signal peptide at the N-terminal, seven N-link glycosylation sites, numerous potential O-linked glycosylation sites in a central mucin-like domain, two glycosaminoglycan attachment sites, five potential hyaluronan-binding motifs, two epidermal growth factor-like domains, and a hydrophobic stretch of 23 amino acids near the C-terminal.

RESULTS: Comparison of the genomic structure of mouse and human SPACRCAN showed significant structure conservation. Analysis of the promoter region revealed several important putative regulatory elements including a Ret-1/PCE-1 element, an 11 base motif for Crx binding, six copies of PIRE, a Ret-4 element, three copies of AP-1, a CRE element, and five copies of GATA3. Northern blot analysis and immunohistochemistry were used to determine the tissue specificity of Spacrcan mRNA and to localize SPACRCAN in developing retina. Spacrcan mRNA is expressed in both retina and pineal gland and was detectable as early as embryonic day 15. The protein is first detectable in the IPM at postnatal day 8 where it increases in concert with the extension of photoreceptor inner and outer segments from the outer retinal surface.

CONCLUSIONS: The presence of several unique regulatory elements in the promoter region and characteristic molecular features shared with the orthologue in human and rat suggest an important functional role of SPACRCAN in the IPM. The time of appearance of the SPACRCAN protein during retinal development suggests that this matrix protein may establish the extracellular microenvironment into which photoreceptor outer segments are elaborated.
Hyaluronan in the Mouse Interphotoreceptor Matrix (IPM) Revisited

Mary E. Rayborn, Karen G. Shadrach, Priyadarshini Senanayake, Joe G. Hollyfield

PURPOSE: We previously reported that the mouse IPM is free of hyaluronan (HA), a conclusion based on the absence of IPM staining in this species with a specific probe for HA (Exp. Eye Res. 65: 603-608, 1997). To determine whether HA could be detected with biochemical methods and with a specific HA probe (bHABC) following attempts to remove HA binding partners, the following studies were performed.

METHODS: BALB/c and C57Bl/6J mouse eyes were used. For HA biochemistry, IPM was extracted in pH 8.0 tris buffered saline, the complex carbohydrate precipitated with ethanol, digested with Streptococcal hyaluronidase and chondroitinase ABC. Samples were analyzed with FACE and disaccharide bands compared to authentic standards. For bHABC analysis, retinas were isolated and rinsed with PBS and then fixed in 2.5% glutaraldehyde in phosphate buffer. Paraffin sections were stained with bHABC.

RESULTS: HA disaccharides were present in the IPM extract from the mouse retina, along with disaccharides of unsulfated chondroitin and 6-sulfated chondroitin. bHABC decorated the IPM in the rinsed retinas, with the cones showing heavier labeling than the rods.

CONCLUSION: We conclude that HA is present in the mouse IPM, as evidenced from the presence of HA disaccharides in the IPM extract, and the binding of bHABC to the IPM in the rinsed retinas. The failure of bHABC to decorate HA in the IPM in our previous analysis was probably due to the complete coverage of HA by matrix molecules that saturate the linear HA molecule, preventing attachment of the HA probe. Supported by FFB and NIH-NEI.
Angiotensin II and its Receptor Subtypes in the Human Eye

P. deS Senanayake, S. Miura, S. Karnik, Joe G. Hollyfield

PURPOSE: To evaluate the distribution of Angiotensin II (Ang II) and its receptors in human ocular tissues.

METHODS: Donor eyes were obtained from the Cleveland Eye Bank within 12 hours of postmortem dissected on a chilled tray and the tissues were stored at -80°C. Ang II receptors were characterized and quantified in optic nerve, RPE-choroid complex, retina and ciliary body-iris by competitive membrane binding assays using Ang II, [Sar1 Ile8] Ang II, and the subtype specific antagonists DUP 753 (AT1-specific) and PD123319 (AT2-specific). Ang II in optic nerve, RPE-choroid complex, retina, vitreous, and ciliary body-iris was extracted with chilled HCL-Ethanol and concentrated using Water’s C18 Sep-Paks. Ang II was quantified by RIA.

RESULTS: Ang II receptors were present in the four tissues studied: retina, 12.1 ± 0.3; RPE-Choroid complex, 6.6 ± 1.1; optic nerve, 3.4 ± 0.1; ciliary body-iris, 2.20 ± 0.4 fmol/mg protein (mean ± se; n=3). In the ciliary body-iris the receptors were exclusively AT1, however in the other tissues, both AT1 and AT2 were present. In the retina AT1 was predominant, in the RPE-choroid complex, the percentage of AT1 was higher than AT2. In the optic nerve, the percentage of AT1 and AT2 was comparable. The highest levels of Ang II were in the optic nerve, range, 8 to 819 pg/g (n=12, median 174). Vitreous had the lowest levels range, 3-32 pg/ml (n=27, median 9). The retina (1-367 pg/g, n=19,median =123), RPE-choroid complex (9-271 pg/g, n=12,median = 43), and ciliary body-iris (5-179 pg/g, n=11,median =44), had comparable levels.

CONCLUSIONS: High levels of Ang II and Ang receptors are present in the vascularized ocular tissues. The variability in the levels of Ang II within each tissue may be a reflection of the heterogeneity of peptide expression and/or the accompanying therapeutic regimens. Local Ang II may be involved in blood supply and/or pathological processes such as neovascularization in diabetic retinopathy. Supported by AstraZeneca Inc.
SPACRCAN Binding to Hyaluronan: Molecular and Biochemical Studies

Q. Chen, K.G. Shadrach, Joe G. Hollyfield

PURPOSE: Hyaluronan (HA) is a glycosaminoglycan (GAG) in the interphotoreceptor matrix (IPM). It has been implicated as providing a primary scaffold in the IPM. Some proteins in IPM, such as SPACR and SPACRCAN, have been shown to possess HA binding motifs. Both of them have been demonstrated to bind to HA. However, there is no direct evidence that the binding is through interaction with the HA binding motif. The purpose here is to initiate a study to demonstrate the function of HA motifs in the HA binding identified in mouse SPACRCAN.

METHODS: A short polypeptide fragment of mouse SPACRCAN containing each HA binding motif was subcloned into the pGEX-2TK vector and expressed in E. coli BL21 cells. Proteins purified from the cell extracts were subjected to CPC precipitation analysis in which binding of a cationic detergent, cetylpyridinium chloride, to anionic GAGs like HA leads to co-precipitation of proteins interacting with the GAGs. To demonstrate the binding was HA specific, digestion with a HA-specific hyaluronidase, Streptomyces hyaluronidase, was included in the co-precipitation analysis.

RESULTS: Polypeptides containing four of the HA binding motifs in mouse SPACRCAN have been expressed in E. coli BL21 cells. CPC precipitation analysis showed that the polypeptides were precipitated in the pellets. However, pre-incubation of the polypeptides with Streptomyces hyaluronidase dramatically decreased the amount of peptides precipitated in the pellets.

CONCLUSIONS: The HA binding motif containing polypeptides expressed in E. coli binds to HA. Further mutagenesis studies are in progress to determine the HA binding is through the HA binding motifs.
TIMP-3 Distribution and Content in Bruch’s Membrane and the Choroid is Different in Caucasian and African American Donor Eyes

H. Sakaguchi, K.G. Shadrach, Mary E. Rayborn, Joe G. Hollyfield

PURPOSE: Age-related macular degeneration (AMD) occurs more frequently in lightly pigmented individuals of Northern European extraction than in more heavily pigmented individuals of African extraction. To determine whether specific molecular differences are present in the connective tissue below the RPE of the macula, we defined the distribution and content of TIMP-3 in Bruch’s membrane between age-matched Caucasian and African American donor tissues.

METHODS: Donor eyes used were between 50 and 85 years of age, 11 from African American donors and 12 from Caucasian donors. Bruch’s membrane and choroid from the macula from each donor eye were prepared for immunocytochemistry and Western blotting and differences in immunoreactivity were quantified.

RESULTS: TIMP-3 immunoreactivity was present in broader areas in Bruch’s membrane and connective tissue surrounding the choriocapillaris in the Caucasian samples than was observed in the African American samples. Additionally, quantitation of Western blots indicated that Caucasian tissues show a progressive increase in TIMP-3 content with age, whereas African American tissues show near steady state levels over the same age ranges.

CONCLUSIONS: TIMP-3 has been proposed to be one of the candidate proteins involved on age-related macular degeneration. Our study suggests that the susceptibility of Caucasians to AMD may be related to the progressive accumulation of proteins in Bruch’s membrane and surrounding tissues that could alter the exchange of metabolites between the RPE and choriocapillaris.
The Human Interphotoreceptor Matrix Proteome—Initial Results

Joe G. Hollyfield, Karen G. Shadrach, Karen A. West, J. Sun, John W. Crabb

PURPOSE: The interphotoreceptor matrix (IPM) serves important roles in retinal physiology yet less than a dozen IPM proteins have been documented to date. Additional proteins must function in this critical retinal extracellular matrix and we have initiated efforts to define the IPM proteome.

METHODS: Human IPM was isolated from dissected retina and posterior eye cup containing RPE by sequential rinses with PBS pH 7 and extractions with TBS at pH 8. Four IPM fractions were obtained: 1) the retina rinse and 2) the eye cup/RPE rinse containing readily soluble IPM components; 3) the retina extraction and 4) eye cup/RPE extraction containing less soluble IPM components. Extracted IPM samples were digested with Chondroitinase ACII to remove chondroitin sulfate-type GAGs from proteoglycans. Proteins were separated by SDS-PAGE, excised from the gel, digested in situ with trypsin and identified by LC MS/MS sequence analysis using Swiss Pro and NCBI databases.

RESULTS: Over 100 different proteins have been identified from initial analyses of the IPM, including interphotoreceptor retinoid-binding protein, SPACR and SPACRCAN. Particularly striking is the number of hypothetical and unknown proteins, accounting for about 15% of these initial protein identifications.

CONCLUSIONS: Proteomic methods are revealing the identity of proteins in the IPM. Hypothetical and unknown proteins may be possible candidate genes for inherited retinal disease. Complete definition of the IPM proteome will lead to a better understanding of the molecular interactions taking place in this important compartment supporting photoreceptor and RPE functions.
Annexins in Retina–Choroid Complex: Gene Expression and Protein Distribution

PURPOSE: The annexins constitute a family of calcium-dependent phospholipid-binding proteins. Several annexins have been identified in our proteomic studies of drusen. To localize the distribution of annexins I–VI in the retina–choroid complex of human eyes, we pursued Western and immunocytochemical analyses using a series of anti-annexin antibodies and also conducted RT-PCR.

METHODS: The ocular tissues analyzed were retina, retinal pigment epithelium, and Bruch’s membrane / choroid. RT-PCR and western blots were performed for the demonstration of annexins. Tissues from human eyes embedded in paraffin were used for immunohistochemistry. Commercially available annexin antibodies were used for light microscopy employing the ABC method.

RESULTS: All annexins studied except annexin-III, were localized using immunocytochemistry throughout the retina, in the RPE, Bruch’s membrane / choroid. Annexins I and II were expressed more strongly in Bruch’s membrane and choroid than in the retina and RPE. Annexins IV, V and VI were expressed more strongly in retina than in the RPE, Bruch’s membrane and choroid.

CONCLUSIONS: All the annexins we studied except annexin III are present in RPE, choroid and retina. Although annexin function is still not clearly defined, detected annexins exhibit a characteristic expression pattern in the retina–choroid complex.
Nuclear Export of TIP120A in Retinal Pigment Epithelium


PURPOSE: TIP120A is reported to be a nucleus transcription factor, but little has been investigated about TIP120A in retinal pigment epithelium (RPE). We have previously reported the presence of TIP120A in RPE cells, and more recently we have recognized TIP120A in the interphotoreceptor matrix, the extracellular space facing RPE cells. We have also discovered that TIP120A is localized in the cytoplasm as well as in the nucleus in RPE cells, while it is generally localized only in the nucleus of non-RPE cells. In this study, we investigate the capacity of TIP120A to export from the nucleus to the cytoplasm or to shuttle between these compartments.

METHODS: Human donor eye tissue and transformed RPE-type cell lines (RPE-J cells and ARPE-19 cells) were analyzed by RT-PCR for TIP120A mRNA. To determine the biochemical localization of TIP120A, subcellular fractionation of RPE cells was performed, and then the fractionated cell samples were blotted for TIP120A. Immunohistochemistry for TIP120A was also done on donor ocular tissue containing RPE cells and cultured RPE cells.

RESULTS: The mRNA for TIP120A is clearly present in human donor RPE cells as well as transformed RPE cell lines. Western blotting for TIP120A also indicated that TIP120A protein exists in RPE cell lines. Immunohistochemistry data showed that TIP120A is localized not only in the nucleus but also in the cytoplasm in donor RPE cells and RPE culture cell lines. Furthermore, it was shown that the deduced amino acid sequence of TIP120A possesses three potential nuclear export signals.

CONCLUSIONS: The present data suggests that TIP120A is a nuclear export-type transcription regulating molecule, and that it is exported from the nucleus to the cytoplasm. Such export or shuttling of TIP120A may be a unique feature of RPE cells. Additional studies are planned to determine the mechanism underlying the nuclear export of TIP120A in RPE cells.
Glycogen in RPE and Choroid of the Diabetic Rat
P.deS. Senanayake, E. Rungger-Brandle, A.A. Dosso, Joe G. Hollyfield

PURPOSE: To evaluate steady state levels and storage of glycogen in RPE-choroid complex after streptozotocin injection.

METHODS: RPE-choroid complex was isolated from pigmented Long Evans rats at 4 and 12 days after induction of diabetes, digested with proteinase K and double ethanol precipitated. Supernatant and precipitate fractions were either 2-aminoacridone (AMAC) derivatized directly to identify endogenous saccharides such as free glucose with free aldehydes or digested with glycoamylase (to determine total glycogen). The digestion products were fluorotagged with AMAC and separated by electrophoresis. The bands were digitized and their intensities quantified. For EM histochemical demonstration of particulate glycogen (glycosomes), thin sections were stained by the periodic acid-thiocarbohydrazide-silver proteinate method.

RESULTS: The levels of glucose, total glycogen and the ratio of glycogen to glucose in the fasting state were similar in the two groups 4 weeks after the induction of diabetes. In the fed state, glucose and total glycogen was higher in the diabetic rats, while the ratio of glycogen to glucose remained the same. At 12 weeks post induction in the fed state, glucose and total glycogen were higher in the diabetic rats but the ratio of glycogen to glucose was lower than the non diabetic rats. Particulate glycogen is present in choroidal fibroblasts and smooth muscle cells in the controls and is massively enriched in the diabetic tissue as early as one week postinjection. By contrast, virtually no glycosomes are detectable in RPE and the endothelium of the choriocapillaris.

CONCLUSION: Our data shows the progression from normal to altered glucose and total glycogen metabolism after the induction of hyperglycemia. Glycogen content in the RPE-choroid complex is increased early on in diabetic animals. The fact that very few glycosomes can be detected in the RPE in hyperglycemic rats suggests that glycogen turnover is rapid and storage minimal. Moreover, our observations purport to cell type-specific stability of glycogen; storage being stable in choroidal fibroblasts and smooth muscle cells, and highly labile in RPE and endothelium.
Glycogen Utilization by Human RPE Cultures
Karen G. Shadrach, P.deS Senanayake, A. Calabro, J.G. Hu, D. Bok, Joe G. Hollyfield

PURPOSE: To evaluate the synthesis of glycogen by confluent retinal pigment epithelium (RPE) cultures that have established high resistance junctions.

METHODS: Human RPE was cultured in Millicell-[PFC] culture plates in medium containing 1 mg/ml glucose and the medium was changed every three days. Efficiency of separation of the apical and basal compartments was determined by transepithelial resistance measurements. Cell with associated matrices (CM), apical (Am) and (Bm) media were collected 6 h after the addition of medium on day 49, digested with proteinase K and ethanol precipitated. Supernatant and precipitate fractions were either 2-aminoacridone (AMAC) derivatized directly to identify endogenous saccharides with free aldehyde or digested with either hyaluronidase SD and chondroitin ABC or alkaline phosphatase (to confirm the identity of phosphate esters) or glycoamylase (to determine total glycogen). The digestion products were flurotagged with AMAC and separated by electrophoresis.

RESULTS: Glucose decreased by 45% in Am and 36% in Bm. Glycogen and derivatives (glucose-6-P, mannose-6-P, glyceraldehyde-3-P, maltose, maltotriose, maltotetraose, unresolved maltooligosaccharides) were present in the CM, maltooligosaccharides were the predominant components. In Am and Bm mannose was predominant, maltose and phosphate esters were also present, and mannose-6-P showed basal polarity. In addition Am and Bm contained unsulfated and sulfated (4S and 6S) chondroitin and it’s secretion was non polar.

CONCLUSIONS: This study demonstrates that the glycogen derivatives and the chondroitins synthesized and secreted by the RPE cultures have a distinct profile compared to the saccharides retained by the CM.
Glycogen, Hyaluronan and Chondroitin Sulfate are Increased in Diabetic Rat Retina

P.deS. Senanayake, E. Rungger-Brandle, A.A. Dosso, Joe G. Hollyfield

PURPOSE: To evaluate steady state levels of glucose, glycogen, hyaluronan and chondroitins in the rat retina after streptozotocin (STZ) injection.

METHODS: Diabetes was induced in pigmented Long Evans rats (200-250g) by i.v. injection of STZ in citrate buffer (60mg/kg body weight). Age-matched control rats were injected with citrate buffer. Hyperglycemia (blood glucose >20 nM ) was detected two days after injection of STZ. Dark-adapted animals were sacrificed 1 and 4 weeks post-injection. The eyes were enucleated and the retina was isolated on a chilled dissecting tray and frozen immediately. The retina was digested based on wet weight with proteinase K and double ethanol precipitated. Supernatant and precipitate fractions were either 2-aminoacridone (AMAC) derivatized directly to identify endogenous saccharides such as free glucose with free aldehydes or digested with hyaluronidase SD, followed by chondroitin ABC and glycoamylase, to identify, characterize and quantitate hyaluronan, chondroitin sulfate and total glycogen. The digestion products were fluorotagged with AMAC and separated by electrophoresis. The bands were digitized and their intensities quantified.

RESULTS: The results showed that the levels of glucose at one and four weeks were five and eleven fold higher, respectively, in the diabetic retina compared to the control. Total glycogen was two and three fold higher at one and four weeks, respectively, post-induction of diabetes. At four weeks post-induction of diabetes, hyaluronan increases three-fold and chondroitin-6 sulfate increases thirteen fold. In contrast, unsulfated chondroitin decreased by six fold.

CONCLUSIONS: From this study, we conclude that: (1) The glycogen metabolism may be perturbed as early as one week after induction of diabetes. (2) Hyaluronan synthesis is increased in the diabetic retina. (3) Sulfation of chondroitin is increased in the diabetic retina. Increased chondroitin-6 sulfate and hyaluronan may produce an expansion of the extracellular matrix of the retina and result in its dysregulation.
Arrest of Phagosome Maturation by Products of Lipid Peroxidation via Inhibition of Phosphoinositide 3-Kinase Recruitment

G. Hoppe, J. O’Neil, H.F. Hoff, Jonathan E. Sears

PURPOSE: Lipid peroxidation has been implicated in many age-related diseases, including macular degeneration of the retina, which is characterized by failure of retinal pigment epithelium (RPE) of the eye fails to process phagocytosed outer segments (OS) of the photoreceptors and accumulation lipofuscin within RPE lysosomes. We sought to elucidate the mechanism, by which accumulation of oxidized lipid-protein crosslinks reduces the ability of RPE cells to process subsequently internalized OS.

METHODS: Primary cultures of human RPE cells were treated with copper-oxidized LDL (oxLDL) as a source of oxidized lipids-protein complexes. Cells were then challenged with magnetic latex beads to initiate phagosome formation, or tested for their ability to internalize and degrade 125I-labeled proteins and OS. Lysosomal enzymatic activity was measured in RPE cellular extracts adjusted to pH 4.5. Isolated phagosomal proteins were analyzed by western blot for the presence of Rab5, EEA1, LAMP-1, cathepsin D, p85, and phosphotyrosine, as well as for the phosphatidylinositol 3-kinase (PI3K) activity.

RESULTS: OxLDL did not reduce the overall lysosomal hydrolytic capacity of the RPE, yet efficiently inhibited processing of various internalized proteinaceous targets. OxLDL caused a delay in the acquisition of lysosomal markers including proteolytic enzymes by newly-formed phagosomes. At the same time, an excessive accumulation of Rab5 and EEA1, markers of early phagosomal compartments, was also observed. The amount of p85, a regulatory subunit of PI3K, was reduced in phagosomes of the RPE treated with oxLDL. This was accompanied by a reduced PI3K activity in the phagosomal fraction. In addition, oxLDL caused a reduction in the phosphotyrosine content of the total cellular protein fraction.

CONCLUSIONS: These results suggest that oxLDL-loading of the RPE prevents phagosome maturation by blocking the recruitment of PI3K to the phagosomal membrane leading to a delayed processing of internalized OS.
Reversal of Protein S-glutathiolation by Glutaredoxin in the Retinal Pigment Epithelium

Y-C Chai, G. Hoppe, Jonathan E. Sears

PURPOSE: Protein cysteines can serve both sensory and activation roles in the regulation of protein function. The modulation of mixed disulfides with glutathione may promise to be a broad mechanism of redox signalling.

METHODS: Using both protein extract and intact RPE cells, we have generated covalent adduction of glutathione to protein cysteines and further show that glutaredoxin (Grx-1) is able to remove glutathione from protein S-glutathiolated substrates.

RESULTS AND CONCLUSIONS: Our data demonstrate that glutathione can modify a wide range of RPE proteins in intact cells, but that the reversal of this process—deglutathiolation and thiol bond restoration—may require a specific catalytic reaction with glutaredoxin. More generally, our experiments support the hypothesis that glutathione can non-specifically become adducted to protein cysteines during oxidative stress, but that the specific, functional reconstitution of protein thiols depends on recognition by an oxidoreductase such as glutaredoxin. This concept offers the idea that redox signalling involves both adduction of a non-specific non-protein reducing equivalent such as glutathione and specific protein based removal by glutaredoxin.
Expression and Localization of Bestrophin During Normal Mouse Development

B. Bakall, L.Y. Marmorstein, G. Hoppe, Neil S. Peachey, C. Wadelius, Alan D. Marmorstein

PURPOSE: Best macular dystrophy is caused by mutations in the VMD2 gene, which encodes the protein bestrophin. The purpose of this study was to determine the postnatal onset of expression of bestrophin mRNA and protein in the mouse retinal pigment epithelium (RPE).

METHODS: Rabbit anti-mouse bestrophin polyclonal antisera designated Pab-003 was generated against a peptide derived from the C terminus of mouse bestrophin and characterized by Western blot and immunofluorescence staining of transfected cells. Expression of bestrophin mRNA during ocular development was studied with quantitative PCR. Bestrophin protein expression in the developing eye was observed by using immunohistochemistry. The onset of mouse phototransduction was determined by conventional electroretinography (ERG).

RESULTS: Bestrophin mRNA was detected at embryonic day 15 in whole mouse eyes by RT-PCR. Real-time quantification of mouse bestrophin mRNA levels indicated that the highest levels of mRNA were present in the early postnatal period. In contrast, bestrophin in the RPE was first detected at postnatal day (P)10 by immunohistochemistry. Phototransduction, as determined by the presence of an ERG a-wave, was first observed at P10.

CONCLUSIONS: The results of this study show that mouse bestrophin mRNA is present in the eye during embryogenesis and significantly precedes the onset of bestrophin protein expression at P10. The appearance of bestrophin in the basolateral plasma membrane of the RPE is coincident with the first detectable ERG a-wave. Because bestrophin is thought to play a role in generating the light peak, a late response of the ERG, these data support a temporal role for bestrophin in RPE responses to light. Furthermore, bestrophin protein appears to be a very late marker of RPE differentiation and to be subject to strong translational control.
Anecortave Acetate Monotherapy for Treatment of Subfoveal Neovascularization in Age-related Macular Degeneration (AMD): Clinical Outcomes at Month 24


PURPOSE: To evaluate safety and efficacy of the angiostatic agent Anecortave Acetate versus placebo for treatment of subfoveal choroidal neovascularization (CNV) due to age-related macular degeneration (AMD).

METHODS: Masked study medication (3 mg, 15 mg, or 30 mg Anecortave Acetate) or placebo (vehicle) was administered as a posterior juxtascleral depot on the outer scleral surface using a unique curved cannula, with additional treatment at 6-month intervals if the masked investigator believed the lesion could benefit. Patients received periodic detailed ophthalmic examinations with angiography, general physical examinations, and hematology/serum chemistry/urinalysis. Safety data were periodically reviewed by an Independent Safety Committee.

RESULTS: At Month 24, Anecortave Acetate 15 mg is statistically superior to placebo and numerically superior to the other concentrations tested in eyes with predominantly classic lesions for visual acuity preservation (p = .004), vision stabilization of vision (less than 3 logMAR line change from baseline) (p = .023), prevention of severe vision loss (decrease of at least 6 logMAR lines) (p = .023), and inhibition of classic CNV growth (p = .011). Superiority of Anecortave Acetate 15 mg versus placebo was also demonstrated in the overall analysis of all treated eyes for these parameters. No safety issues related to either the administration procedure or the study medication were observed.

CONCLUSIONS: Posterior juxtascleral administration of Anecortave Acetate 15 mg continues to be clinically superior to placebo at Month 24 in this safety and efficacy study for protection of vision and suppression of lesion growth. These long-term results confirm results at Months 6 and 12.
Optical Coherence Tomography Evaluation in Age-related Macular Degeneration

Peter K. Kaiser

PURPOSE AND METHODS: It is possible with OCT to directly visualize chorioidal neovascularization (CNV) providing a structural rather than a functional assessment of neovascularization. This is useful in deciding if the CNV is type I (above the RPE) or type II (below the RPE). We use OCT as a step in determining a patient’s eligibility for submacular surgery especially in younger patients with CNV due to ocular histoplasmosis syndrome, punctate inner choroidopathy, multifocal choroiditis, or pathological myopia.

RESULTS AND CONCLUSIONS: Since OCT can effectively quantify neurosensory and pigment epithelial detachments and localize subretinal versus intraretinal fluid accumulation, it has improved our understanding of the retinal changes after ocular photodynamic therapy. We use OCT to help guide re-treatment decisions in cases where the fluorescein angiogram is equivocal. For example, patients with stable visual acuity, fluorescein leakage on angiography, but evidence of fibrosis or absence of fluid on OCT can be observed without retreatment. In contrast, patients with stable visual acuity, fluorescein leakage on angiography, and fluid on OCT are retreated with ocular photodynamic therapy. This aspect of OCT imaging is still undergoing refinement and will undoubtedly increase in the future. As with fluorescein angiography, limitations exist in the use of OCT in the assessment of CNV. For example, OCT is unable to detect CNV beneath serous pigment epithelial detachments because of optical shadowing and the absence of a detectable reflection from the RPE/choriocapillaris. Although OCT in its current configuration is useful in enhancing the assessment of CNV and possibly guiding re-treatment decisions, it is not a substitute for fluorescein angiography in evaluating or treating CNV.
Five-Year Results of Verteporfin Therapy for Subfoveal CNV Due to AMD: Third Year of an Open Label Extension of the TAP Investigation

Peter K. Kaiser, The TAP Study Group

PURPOSE: To report 5-year results from the Treatment of AMD with Photodynamic therapy (TAP) Investigation that include 3-year results from an open-label extension evaluating verteporfin therapy (Visudyne, Novartis AG) in AMD patients with classic-containing subfoveal CNV for vision outcomes of patients with predominantly classic lesions and safety outcomes for all participants.

METHODS: Patients who completed 24 months of the TAP Investigation and who it was judged might benefit further from verteporfin therapy, were enrolled into the TAP Extension. Methods were similar to those in the TAP Investigation, except extension patients with fluorescein leakage from CNV received open-label verteporfin therapy irrespective of their treatment assignment (verteporfin or placebo) at baseline.

RESULTS: The enrolled participants included 124 (78%) of the original 159 verteporfin-treated patients with lesions composed of predominantly classic CNV at baseline, of whom 93 (58%) of the 159 completed the month 48 examination. Visual acuity measurements were done for 92, 90, and 93 patients at month 24, 36, and 48, respectively. A loss of $\geq 15$ letters of visual acuity from baseline occurred in 33 (36%) at month 36, and 40 (43%) at the month 48 examination, with a mean letter score loss of 8.7, 9.9, and 10.4, respectively. During the extension no additional safety concerns were noted in any patient receiving verteporfin. Two patients originally assigned to placebo had acute severe vision decrease (loss of $\geq 20$ letters of visual acuity within 7 days of treatment) in the study eye between months 24 and 36. No additional case of acute severe vision decrease was reported between months 36 and 48. Vision and safety outcomes through the month 60 examination will be presented.

CONCLUSIONS: Vision outcomes remained relatively stable for verteporfin-treated patients with predominantly classic lesions from the month 24 to the month 48 examination. Caution in the interpretation of these results appears warranted in the absence of a comparison with an untreated group during the extension and because not all patients in the TAP Investigation participated in the TAP Extension.
OCT Characteristics, Correlations, and Classification of Diabetic Macular Edema

B.Y. Kim, Jonathan E. Sears, Peter K. Kaiser

PURPOSE: To describe the various morphological patterns of diabetic macular edema demonstrated by optical coherence tomography (OCT).

METHODS: A retrospective chart review of all patients with clinically-evident diabetic macular edema who underwent OCT evaluation at the Cole Eye Institute between May 1998 and August 2001 was performed. The OCT scans were evaluated for the presence of retinal thickening, cystoid macular edema, hyaloidal traction, serous retinal detachment, and traction retinal detachment. In addition, the foveal retinal thickness was measured.

RESULTS: A total of 199 eyes from 144 patients were identified. OCT revealed at least five distinct morphologic subgroups of diabetic macular edema: diffuse retinal thickening (192 [96%]), cystoid macular edema (114 [57%]), subretinal fluid without posterior hyaloidal traction (21 [11%]), posterior hyaloidal traction without traction retinal detachment (28 [14%]), and posterior hyaloidal traction with traction retinal detachment (3 [2%]). The patterns were not mutually exclusive, and occurred in the following combinations: diffuse retinal thickening combined with cystoid macular edema (64 of 199 scans, 32%); diffuse retinal thickening, cystoid macular edema, and subretinal fluid (17, 8.5%); diffuse retinal thickening, cystoid macular edema, and posterior hyaloidal traction (16, 8.0%); diffuse retinal thickening and posterior hyaloidal traction (11, 5.5%); diffuse retinal thickening, cystoid macular edema, posterior hyaloidal traction and traction retinal detachment (2, 1%); and diffuse retinal thickening, posterior hyaloidal traction, and traction retinal detachment (1, 0.5%). The mean retinal thickness varied within each subtype: diffuse retinal thickness averaged 411.0 ± 132.9 microns (range 215-772 microns), cystoid macular edema 473.7 ± 126.0 microns (235-772), subretinal fluid without posterior hyaloidal traction 547.1 ± 93.0 microns (376-760), posterior hyaloidal traction without traction retinal detachment 448.9 ± 123.9 microns (255-765), and posterior hyaloidal traction with traction retinal detachment 576.8 ± 124.3 microns (376-759).

CONCLUSIONS: Diabetic macular edema exhibits at least five different morphologic patterns on optical coherence tomography with varying incidence rates.
Intravitreal Triamcinolone Acetonide for Refractory Uveitic Cystoid Macular Edema

Michelle L. Young, Peter K. Kaiser, Careen Y. Lowder

PURPOSE: To evaluate the retinal structures, retinal thickness, and visual acuity before and after intravitreal triamcinolone acetonide injection for refractory uveitic cystoid macular edema.

METHODS: Prospective, interventional, consecutive case series of patients with refractory cystoid macular edema secondary to uveitis seen at the Cole Eye Institute. Thirteen eyes of eleven patients with uveitis and cystoid macular edema involving the center of the fovea received an intravitreal injection of 4 mg triamcinolone acetonide in the inferior quadrant after failing previous posterior sub-Tenon’s triamcinolone acetonide injection(s). Best corrected visual acuity, intraocular pressure, clinical examination, and optical coherence tomography (OCT) scans were performed at baseline, one and three months post-injection.

RESULTS: Thirteen eyes of eleven patients were identified. Baseline OCT scans demonstrated retinal thickening and cystoid macular edema in all eyes. Mean baseline retinal thickness was 479 microns (range, 353 to 606 microns). Mean post-injection retinal thickness significantly decreased, averaging 238 microns (range, 130 to 395 microns; p=0.0006). In all cases there was a decrease in the amount of cystic spaces seen on OCT. Median follow up was 13 weeks. Mean visual acuity improved from 20/95 to 20/54 (p=0.0653). No patients experienced a decrease in vision. There were no cases of increased intraocular pressure or ocular complications.

CONCLUSION: OCT demonstrated a significant improvement in retinal thickening and decreased cystic changes after intravitreal triamcinolone acetonide injection for refractory uveitic cystoid macular edema. Visual acuity also improved suggesting intravitreal triamcinolone acetonide injections may be effective in the treatment of uveitic cystoid macular edema.
Posterior Juxtascleral Delivery of Anecortave Acetate for Subfoveal CNV – Clinical Safety and Feasibility of a Unique Route of Administration


PURPOSE: To evaluate the clinical safety and feasibility of administering anecortave acetate onto the outer scleral surface as a posterior juxtascleral depot using a specially designed cannula.

METHODS: To date, this unique trans-scleral route of administration has been used in five clinical trials evaluating anecortave acetate for the treatment of subfoveal CNV secondary to AMD. Clinical safety results from two of these five studies after 6 to 36 months of treatment have been reviewed by the Independent Safety Committee overseeing these activities. In the ongoing 24-month dose-response study, patients were randomized to anecortave acetate or placebo treatment, with optional re-treatment at 6-month intervals. In a completed 6-month study, efficacy of a single administration of anecortave acetate versus placebo was assessed in patients receiving their initial Visudyne PDT® treatment. The systemic clinical safety of anecortave acetate was assessed in both studies by periodic general physical examinations and evaluations of blood chemistry/CBC/urinalysis. The ophthalmic safety was assessed by periodic detailed ophthalmic examinations.

RESULTS: To date, across all 5 studies, 454 patients have received a total of 697 posterior juxtascleral administrations of anecortave acetate or placebo. Multiple re-treatments have been successfully performed through small incisions into the same superotemporal quadrant of the orbit. All adverse events reported to date were typically mild, transient, observed in all treatment groups, and assessed as unrelated to study medication. Adverse events possibly related to the injection technique include ptosis, ocular pain and subconjunctival hemorrhage.

CONCLUSIONS: A unique posterior juxtascleral procedure using a curved cannula for the trans-scleral depot delivery of anecortave acetate to the choroid and retina of AMD patients has been developed and used in five clinical trials. This delivery procedure has proved to be safe during both single and repeated treatments, and no clinically relevant adverse events related to either anecortave acetate or the administration procedure have been identified.
Ocular PDT Registry

Peter K. Kaiser

PURPOSE: To use an Internet-based, secure, password-protected database to follow the outcomes of patients with choroidal neovascularization treated with verteporfin photodynamic therapy.

METHODS: After signing an Institutional Review Board approved-informed consent, patients undergoing verteporfin photodynamic therapy (PDT) for choroidal neovascularization are entered into an Internet-based, secure, password-protected database. Baseline demographics and follow-up data including age; sex; cause of CNV; previous treatments; size, location, and percent classic of the CNV; lesion components; use of adjuvant therapies; and PDT parameters are entered into the database.

RESULTS: 737 patients with AMD received PDT. Mean baseline visual acuity (VA) was 20/200-1, and lesion size was 3470 microns. After a mean of 308 days, PDT treatment was given 2.6 times. The mean change in VA from baseline was +5.3 letters in the poor VA group (≤20/200) and −11.9 letters in the good VA group at 118 days follow up and +4.8 letters in the good VA group and −13.8 letters in the poor VA group at the 308 day follow up evaluation.

CONCLUSIONS: Baseline vision was worse while the lesion size was larger than the TAP Study. Patients with poor baseline VA (≤20/200) had a mean VA improvement of 1 line; however, the patients with similar baseline VA as the TAP Study had approximately 1 line more visual acuity loss at 1 year follow up. One explanation for this difference is the lower number of treatments received in this analysis (2.6) as compared to the TAP Study (4.0).
Evaluation of the DORC and Alcon 25-gauge System with the Alcon Accurus Vitrectomy Machine

Peter K. Kaiser

PURPOSE: To evaluate the DORC and Alcon 25 gauge vitrectomy system with the Alcon Accurus™ vitrectomy machine.

METHODS: The DORC and Alcon 25 gauge (g) trocars, infusion cannula, and vitrectomy probes, as well as the DORC 25g extrusion backflush handle (passive or active), scissors, picks, and forceps were used in a variety of vitreoretinal surgical procedures including macular holes, epiretinal membranes, vitreous hemorrhage, diabetic macular edema due to posterior or hyaloidal traction, vitreomacular traction syndrome, retinal detachment, and endophthalmitis cases in combination with the Alcon Accurus™ vitrectomy machine. Patients with complicated tractional diabetic detachments, proliferative vitreoretinopathy, or cases that would require 20g instruments were excluded. Any problems and observations were recorded during and after the surgeries.

RESULTS: The 25g trocars and infusion cannula were inserted/removed through the sclera and transconjunctively very easily, making the “opening/closing” almost instantaneous. The infusion cannula was a little loose in the trocar, but did not spontaneously eject even under high infusion pressures. Both the DORC and Alcon lightweight 25g pneumatic probes had excellent ergonomics and were easily rotated and manipulated in either hand. With low infusion pressures, it was possible to collapse the eye; however, by increasing infusion rates (50-70 mmHg; up to 90 with 1st generation) the pressure maintenance was judged to be “good to excellent.” The infusion rates were quickly decreased to normal (20-30 mmHg) when no longer aspirating with the Accurus™ software/footswitch control. Finally, the probes offered precise control of cutting especially at high speeds (up to 1500 cpm) near the retinal surface with good flow rates. The use of traditional 20g instruments was not required in any case. Examination of the sclera (in the early cases where a conjunctival peritomy was performed) failed to reveal any leakage at the sclerotomy sites even with extensive movement of the eye during the case. No patients had hypotony during the post-operative period. Patients anecdotally had less complaints of ocular discomfort compared to 20g post-op patients, and less post-op inflammation.

CONCLUSIONS: The use of a 25 gauge vitrectomy system improves surgical efficiency and appears to be well tolerated by patients. However, it cannot be used in all cases, requires a small learning curve, and not all instruments are available, so case selection is important.
Sterile, Disposable, Lighted, Multifunction; Grieshaber Revolution DSP Instruments for Vitreoretinal Surgery

Peter K. Kaiser

PURPOSE AND METHODS: The Grieshaber Revolution™ DSP system consists of disposable, pre-assembled, sterile, single use scissors and fiber optic forceps that offer convenience and a high level of performance for vitreoretinal surgery. One of the most unique features of this system is that the forceps tip is constructed of fiber optic material that simultaneously delivers both illumination that eliminates annoying shadows, inadequate light intensity, instrument glare and the many inconveniences of setting up typical multi-function fiber optic instruments, while also providing a stable grasping platform. The integrated fiber in the forceps is easily connected to the existing light source of the vitrectomy machine and is especially useful in complicated diabetic traction detachment and proliferative vitreoretinopathy cases.

RESULTS AND CONCLUSIONS: Since the instruments are sterile, disposable, and single use, facilities that perform infrequent vitrectomies will eliminate the need for a qualified instrumentation staff to maintain and process these delicate instruments, thereby offering significant cost reduction of sterile processing. Moreover, the sterile barrier packaging eliminates cross-contamination concerns especially in the light of CJD, HIV and Hepatitis C. In addition, most hand-held instruments must be sent for repair when damaged or broken due to instrument fragility and improper handling. These instruments eliminate this problem. Finally, since capital expenditures are not required, the Grieshaber Revolution DSP is ideal for hospital OR’s, Ambulatory Surgery Centers, satellite clinics and smaller/rural community hospitals.
Acute Endophthalmitis Following Intravitreal Triamcinolone Acetonide Injection


PURPOSE: To report the clinical features of 7 patients who developed acute endophthalmitis following intravitreal injection of triamcinolone acetonide.

METHODS: A retrospective, interventional case series of all patients at 4 clinical centers presenting with acute endophthalmitis following intravitreal triamcinolone acetonide (Kenalog) injection was performed between March 2001 and March 2002. At the 4 centers, all intravitreal injections were performed using sterile techniques followed by a postoperative regimen of topical antibiotics. The following data were collected: indication for injection, predisposing factors, time to presentation, clinical findings, additional procedures, presenting and final visual acuities, culture results, type of Kenalog bottle used, injection technique, and length of follow-up.

RESULTS: Seven eyes of 7 patients were identified: 6 patients were Caucasian, 6 cases involved the left eye, and 4 patients were female. The median age was 61 years (range, 26–73 years). Indications for treatment included refractory cystoid macular edema (n=3), refractory clinically significant diabetic macular edema despite laser treatment (n=2), and choroidal neovascular membrane (n=2). Predisposing risk factors included filtering blebs (n=2), non-insulin dependent diabetes mellitus (n=2), and injection from a multi-use Kenalog bottle (n=4). The median time to presentation was 4.0 days (range, 1–10 days). The most common clinical findings were red eye (n=2), blurry vision (n=3), decreased vision (n=3), hypopyon (n=4), iritis (n=7), and vitritis (n=7). Only one patient complained of eye pain. The median presenting visual acuity was 20/400 (range, 20/60 to Light Perception). Treatment consisted of vitreous tap and injection of antibiotics (n=4), pars plana vitrectomy and injection of intravitreal antibiotics (n=1), and systemic treatment alone with oral levofloxacin (n=2). In addition, 4 patients were treated with topical antibiotics. Cultures were obtained in 5 patients: 4 were sterile, and 1 grew out mycobacterium chelonae. Only one patient required additional procedures (The patient with m. chelonae) requiring 3 subsequent vitreous tap and injections, and 3 pars plana vitrectomies with final vision of NLP. After a median follow-up of 1.8 months (range, 0.8–5.0 months), 4 patients demonstrated an improvement in visual acuity, two had stable vision, and one had a decrease in vision. The median post-infection vision significantly improved to 20/80 (range, 20/40 to No Light Perception)(p < 0.05).

CONCLUSIONS: Acute endophthalmitis following intravitreal kenalog injection occurs rapidly and can result in severe loss of vision; however, early recognition and aggressive treatment can result in good visual outcomes.
Acute Postoperative Endophthalmitis Following IVTA


PURPOSE: To report the clinical features, causative organisms, management, and visual acuity outcomes of eight eyes of eight patients who developed acute postoperative endophthalmitis following intravitreal injection of triamcinolone acetonide (IVTA). DESIGN: Retrospective, multicenter, interventional, case series.

METHODS: A retrospective, interventional, case series of all patients with acute postoperative endophthalmitis following IVTA at seven academic clinical centers between March 2001 and July 2002.

RESULTS: A total of 922 IVTAs were performed. Eight eyes of eight patients with acute postoperative endophthalmitis were identified in the 6 weeks following IVTA for an incidence of 0.87% (95% confidence interval of 0.38% to 1.70%). The median time to presentation was 7.5 days (range, 1–15 days) after IVTA. The most common clinical findings were iritis (n = 8), vitritis (n = 8), hypopyon (n = 8), pain (n = 7), red eye (n = 6), and decreased vision (n = 5). The median presenting visual acuity was 20/1127 (range, 20/60 to light perception). Initial treatment consisted of vitreous tap and injection of antibiotics (n = 6) or pars plana vitrectomy and injection of intravitreal antibiotics (n = 2). Intraocular cultures yielded identification in seven patients. One demonstrated intracellular gram-positive cocci in chains with numerous polymorphonuclear cells on gram stain. The median postinfection vision was 20/400 (range, 20/40 to no light perception). Three patients ended up with no light perception visual acuity, including enucleation (n = 1) and phthisis (n = 1).

CONCLUSIONS: Acute postoperative endophthalmitis following IVTA occurs rapidly and can result in severe loss of vision.
Occlusive Vasculitis Secondary to West Nile Virus

Peter K. Kaiser, Michael S. Lee, D. Martin

PURPOSE: To describe a patient with occlusive, retinal vasculitis and concomitant, confirmed, acute West Nile virus (WNV) infection. DESIGN: Observational case report.

METHODS: Main outcome measures included comprehensive ophthalmic examination with fluorescein angiography, color photography, and serologic testing for WNV and St. Louis encephalitis (SLE) virus including plaque reduction neutralization testing (PRNT).

RESULTS: A 46-year-old woman developed a sudden decrease in vision in her left eye 2 weeks after confirmed WNV infection and demonstrated multiple, small, patchy areas of retinal edema with scattered microaneurysms. Fluorescein angiography showed multiple branch artery occlusions with extensive nonperfusion. Serologic titers for WNV were positive for acute infection. Plaque reduction neutralization testing confirmed WNV infection and excluded St. Louis encephalitis virus infection. Other etiologies of occlusive vasculitis were not present.

CONCLUSIONS: Occlusive, retinal vasculitis may occur in the setting of acute WNV infection.
Retinal Cone Toxicity in an Ovarian Cancer Patient Treated with Irofulven

Michael S. Lee, N. Gupta, J. Loewenstein, M. Wepner, A.M. Milam

PURPOSE: Four of 19 patients at one center with platinum-resistant, advanced epithelial ovarian cancer in a phase II clinical trial using high doses of Irofulven developed signs and symptoms consistent with retinal cone dysfunction. Each complained of photophobia and reduced vision in bright light, while 3 noted various positive visual phenomena. ERG results revealed predominantly cone abnormalities. Improvement in symptoms, visual function testing, and ERG responses occurred with lower doses or cessation of Irofulven. One woman, who received cisplatin prior to entering the trial and carboplatin afterwards, developed AML 10 weeks after her visual symptoms began and died. We describe the clinical, perimetric, electroretinographic, retinal histopathology and immunocytochemistry features of this patient.

METHODS: The patient underwent comprehensive neuro-ophthalmic evaluation including Goldmann visual fields and electroretinography. Post mortem eyes of the patient and age matched normal human eyes were processed for histopathology and immunocytochemistry. Mouse monoclonal antibodies specific for cones and rods and a rabbit polyclonal antibody against glial fibrillary acidic protein (GFAP; specific for astrocytes and reactive Müller cells) were used.

RESULTS: The visual acuities were 20/30 and she identified 3/10 Ishihara color plates OU. Goldmann visual fields revealed dense midperipheral and paracentral scotomas. Dilated fundus examination was unremarkable and carcinoma associated retinopathy testing was negative. Two months later her vision off Irofulven was 20/30 OD; 20/25 OS and 10/10 color plates. The ERG was extinguished under bright photopic conditions and 30 Hz flicker testing revealed markedly low b-wave amplitudes and prolonged implicit times. Scotopic conditions showed prolonged implicit times and borderline b-wave amplitudes. Compared to normal retinas the patient’s retina had reduced numbers of macular cones and almost no cones in the peripheral retina. Near normal numbers of rods were present in all regions examined. Müller cells had undergone reactive gliosis and were positive for GFAP, consistent with retinal cone cell death.

CONCLUSIONS: High dose Irofulven may cause cone specific retinal toxicity with relative sparing of rods.
Identification of the Gene and the Mutation Responsible for the Mouse Nob Phenotype

R.G. Gregg, S. Mukhopadhyay, S.I. Candille, S.L. Ball, M.T. Pardue, M.A. McCall, Neil S. Peachey

PURPOSE: The available evidence indicates that the naturally occurring mouse mutant nob (no b-wave) provides an animal model for the complete form of human X-linked congenital stationary night blindness (CSNB1). The goals of the present study were to identify the nob gene defect, to characterize the expression pattern of the involved gene, and to assess visual sensitivity in nob mice.

METHODS: Positional cloning, screening of candidate genes, and sequencing were used to identify the nob gene. The expression pattern of the nyx gene was examined with Northern blot analysis and in situ hybridization. Visual sensitivity was measured with an active avoidance behavioral test.

RESULTS: The nob phenotype is caused by an 85-bp deletion in the mouse nyx gene, which encodes the nyctalopin protein. Expression of nyx was most abundant in the retina and, in particular, in the inner nuclear layer. The nyctalopin protein contains 11 leucine-rich repeats and is flanked by cysteine rich regions, which identifies it as a member of the small leucine rich proteoglycan family. Behavioral testing shows that nob mice have a significant decrease in visual sensitivity.

CONCLUSION: The nob mouse is a model for human CSNB1. This model will be useful in defining the role of nyctalopin in signal transmission between photoreceptors and retinal bipolar cells.
Expression and Localization of Bestrophin During Normal Mouse Development

B. Bakall, L.Y. Marmorstein, George Hoppe, Neil S. Peachey, C. Wadelius, Alan D. Marmorstein

PURPOSE: Best macular dystrophy is caused by mutations in the VMD2 gene, which encodes the protein bestrophin. The purpose of this study was to determine the postnatal onset of expression of bestrophin mRNA and protein in the mouse retinal pigment epithelium (RPE).

METHODS: Rabbit anti-mouse bestrophin polyclonal antisera designated Pab-003 was generated against a peptide derived from the C terminus of mouse bestrophin and characterized by Western blot and immunofluorescence staining of transfected cells. Expression of bestrophin mRNA during ocular development was studied with quantitative PCR. Bestrophin protein expression in the developing eye was observed by using immunohistochemistry. The onset of mouse phototransduction was determined by conventional electroretinography (ERG).

RESULTS: Bestrophin mRNA was detected at embryonic day 15 in whole mouse eyes by RT-PCR. Real-time quantification of mouse bestrophin mRNA levels indicated that the highest levels of mRNA were present in the early postnatal period. In contrast, bestrophin in the RPE was first detected at postnatal day (P)10 by immunohistochemistry. Phototransduction, as determined by the presence of an ERG a-wave, was first observed at P10.

CONCLUSIONS: The results of this study show that mouse bestrophin mRNA is present in the eye during embryogenesis and significantly precedes the onset of bestrophin protein expression at P10. The appearance of bestrophin in the basolateral plasma membrane of the RPE is coincident with the first detectable ERG a-wave. Because bestrophin is thought to play a role in generating the light peak, a late response of the ERG, these data support a temporal role for bestrophin in RPE responses to light. Furthermore, bestrophin protein appears to be a very late marker of RPE differentiation and to be subject to strong translational control.
Immunohistochemical Analysis of the Outer Plexiform Layer in the Nob Mouse Shows No Abnormalities

S.L. Ball, M.T. Pardue, M.A. McCall, R.G. Gregg, Neil S. Peachey

PURPOSE: In the nob mouse, a mutation in nyctalopin results in a loss of signal transmission from photoreceptors to depolarizing bipolar cells (DBCs).

METHODS: We used immunohistochemical techniques to assess the expression pattern of proteins found at either the photoreceptor terminal or bipolar cell dendrites within the outer plexiform layer. We labeled normal and nob retinas with antibodies against mGluR6, PKC, G0alpha, bassoon, PSD-95, the alpha1F subunit of voltage-gated calcium channels, trkB, and dystrophin.

RESULTS: All labeling patterns in nob and normal retinas were comparable to those previously reported in mouse retina.

CONCLUSION: Our results indicate that the absence of nyctalopin does not disrupt the expression pattern of other proteins known to be required for synaptic transmission.
Neuroprotective Effect of the Subretinal Artificial Silicon Retina

M.T. Pardue, S.L. Ball, H. Yin, M.J. Phillips, Neil S. Peachey, B. Hanzlicek, B. Sippy, A.Y. Chow

PURPOSE: To evaluate possible neuroprotective effects of the subretinal artificial silicon retina (ASR) by measuring retinal function and photoreceptor preservation in RCS rats implanted at an early stage of degeneration.

METHODS: Three week old RCS rats were implanted with an active ASR in one eye while the other eye was implanted with an inactive ASR, underwent a sham surgery, or served as an unoperated control. Retinal function was measured with ERGs before and after implantation. The number of photoreceptor nuclei were measured in plastic sections obtained from a subset of rats that were euthanized at 8 weeks post-implantation.

RESULTS: While retinal function declined in all groups, preservation of retinal function occurred in eyes implanted with the ASR. Eyes implanted with the active ASRs showed significantly larger dark-adapted b-wave amplitudes than eyes implanted with inactive ASRs, sham operated or unoperated. In comparison, the dark-adapted a-wave decreased in amplitude equally in all groups. Counts of photoreceptor nuclei revealed a significantly larger number of photoreceptors directly overlying the implant in eyes implanted with an active or inactive ASR compared to unoperated or sham controls. However, no significant differences in photoreceptor numbers were detected between the eyes implanted with the active and inactive ASR.

CONCLUSIONS: These results indicate that the subretinal ASR may have a temporary protective effect on the RCS retina. While photoreceptor cell counts at 8 weeks did not reveal a significant difference between active and inactive ASR implanted retinas, the ERG data suggest that electrical stimulation provides a protective effect.
Stimulus–Response Characteristics of the Mouse DC-ERG

J. Wu, Alan D. Marmorstein, Neil S. Peachey

PURPOSE: To characterize ERG components generated by non-neuronal tissues of the mouse eye.

METHODS: After overnight dark adaptation, adult mice (C57BL/6J and BALBc/ByJ) were sedated with ketamine/xylazine and placed on a heating pad. Two Ag/AgCl electrodes were fashioned with capillary tubes filled with Hanks BSS; one was placed in contact with the test eye and the other contacted the fellow eye, which was shielded from light stimulation delivered to the test eye. The dc-ERG signal was amplified (dc-100 Hz), digitized (20 Hz) and stored off-line. Full-field stimuli, 7-min in duration, were presented using a Uniblitz shutter. Flash intensity was controlled with neutral density filters.

RESULTS: In response to a 7-min flash, the mouse dc-ERG included an initial b-wave which was followed by a c-wave, fast oscillation (FO), light peak (LP), and an off-response at flash offset. As flash intensity increased, the c-wave, FO, and LP first increased and then decreased in amplitude. The polarity of the off-response was negative for low intensity stimuli and positive at the high end of the stimulus range; polarity reversal occurred at a lower intensity for BALBc/ByJ than C57BL/6J mice.

CONCLUSIONS: The major components of the dc-ERG are readily measured in the mouse. Therefore, this recording technique may be used to examine the effects of genetic manipulation on the electrical activity of the RPE, and how this is altered in retinal degenerative disorders.
Evaluation of Inner Retinal Activity in Mutant Mice Using c-fos Immunohistochemistry

Brett W. Hanzlicek, Neil S. Peachey, Sherry L. Ball

PURPOSE: Visual information is mediated by multiple pathways in the mouse retina. Mice with functional defects in these pathways provide the opportunity to study their contribution to various aspects of visual function. For example, the nob mouse lacks communication between photoreceptors and depolarizing bipolar cells (DBC) while transducin null mice (Tr-/-) lack rod-mediated function. We have examined how these mutations alter inner retinal activity, by monitoring light-induced expression of an immediate early gene, c-fos, in a subpopulation of amacrine cells.

METHODS: After overnight dark adaptation, mice were exposed to a strobe light stimulus presented at 2 Hz for 60 min. In different trials, light intensity varied from -2.7 to 0.4 log cd/sec m². For each stimulus condition, at least 3 wild-type (WT), nob, Tr-/- and Tr+/- mice were studied. Eyes were removed immediately following light exposure and processed for immunohistochemistry with c-fos anti-serum (Santa Cruz Biotechnology). Using light microscopy, c-fos-positive cells were counted in the inner nuclear layer.

RESULTS: In all mouse lines, the number of cells labeled for c-fos increased with increasing stimulus intensity. Fewer cells labeled for c-fos in Tr-/- or nob mice than in WT or Tr+/- retinas. In mice exposed to the lowest flash intensities, more cells were labeled in nob than in Tr-/- retinas. At the highest flash intensity more cells were labeled in Tr-/- than in nob retinas.

CONCLUSIONS: These results indicate that c-fos activation can be used as an assay of inner retinal function and, when applied to mice lacking particular pathways, to elucidate inner retinal circuitry. Our results also indicate that c-fos activation is predominantly mediated by rod DBCs. Differences between Tr-/- and nob mice may reflect the use of an alternative rod pathway spared by the nob defect.
Effects of Fat-Soluble Vitamins and Cholesterol Supplementation on Retinal Structure and Function in an Animal Model of Smith-Lemli-Opitz Syndrome


PURPOSE: Patients afflicted with Smith-Lemli-Opitz syndrome (SLOS, an autosomal recessive metabolic disease caused by defective cholesterol biosynthesis) have abnormally low levels of cholesterol (Chol) and excessive levels of 7-dehydrocholesterol (7DHC) in all bodily tissues. The sterol metabolic defect is accompanied by inefficient absorption of dietary fat-soluble compounds (e.g., vitamins and sterols). We evaluated the ability of systemically administered fat-soluble vitamins and/or Chol to ameliorate or prevent retinal degeneration in a rat model of SLOS.

METHODS: Pregnant Sprague-Dawley rats (6 days sperm-positive; N=8) were given AY9944 (an inhibitor of the defective enzyme in SLOS) in their diet and their progeny were injected 3X per wk with AY9944 as an aqueous-olive oil emulsion (Fliesler et al., IOVS 40:1792, 1999). In parallel, 2 control groups received vehicle injections only and no drug. Four drug treatment groups were established, according to supplementation of the vehicle with: A) no additions; B) vitamins A, D, and E; C) 2% (w/v) Chol; D) vitamins plus Chol. Rats were maintained under dim cyclic light (12L:12D, 20-40 lux) and fed Chol-free chow and water ad lib. At 9 post-natal weeks, dark- and light-adapted ERGs were recorded; one eye from each rat was taken for histological and quantitative morphometric analysis, while the contralateral retina, as well as serum, liver, and brain, were harvested for sterol analysis.

RESULTS: 7DHC/Chol mole ratio values for retinas from all treatment groups were comparable, and were >500X higher than for control retinas. Rod and cone function were markedly compromised (decreased amplitudes, increased implicit times), relative to controls, and retinal degeneration (particularly photoreceptor loss) was substantial and comparable in all treatment groups.

CONCLUSIONS: Under the given conditions, systemic administration of fat-soluble vitamins (A, D, E) and/or Chol does not ameliorate or prevent retinal degeneration in this animal model of SLOS. Higher concentrations of these supplements, in combination with dietary administration, may be required to overcome the cholesterol biosynthesis defect and reduce the severity of the associated retinal degeneration and dysfunction.
Correlation of Bestrophin Protein Expression in the Basolateral Plasma Membrane of the Mouse RPE with the Onset of Photoreceptor Activity in the Retina

B. Bakall, Neil S. Peachey, L.Y. Marmorstein, C. Wadelius, Alan D. Marmorstein

PURPOSE: Best macular dystrophy is an autosomal dominant disease, caused by mutations in the VMD2 gene that encodes the protein bestrophin. To increase our understanding of the function of bestrophin, the onset of bestrophin expression was followed in mouse eyes during development.

METHODS: BALBc mice were examined at different embryonic (E) and postnatal (P) ages. Bestrophin mRNA was quantified using Taq-man quantitative PCR. Immunohistochemistry for mouse bestrophin was performed on paraffin-embedded sections, using a polyclonal mouse bestrophin antibody produced by immunizing rabbits with a peptide corresponding to the mouse bestrophin C-terminus. Electroretinograms were recorded from the corneal surface of mice beginning at P8, to strobe flashes after overnight dark adaptation.

RESULTS: Bestrophin mRNA was detected as early as E15. Bestrophin protein expression in the RPE was not observed until P10 at which time it was observed to localize to the basolateral membrane of the RPE. The earliest age at which an a-wave could be recorded from mouse eyes was P10, coincident with the onset of bestrophin protein expression in the RPE.

CONCLUSIONS: Despite the presence of mRNA for Bestrophin during embryonic development, the onset of protein expression in RPE is late, beginning at P10. This coincides with the onset of photoreceptor electrical activity, supporting the hypothesis that bestrophin plays a role in the RPE electrical responses to phototransduction. In addition, we conclude that bestrophin protein is a late marker for RPE differentiation.
Endogenous Oxidoreductase Expression is Induced by Aminoglycosides

George Hoppe, Y.C. Chai, Jonathan E. Sears

PURPOSE: Oxidoreductases such as glutaredoxin are a major class of enzymes that reversibly catalyze thiol-disulfide exchange reactions.

METHODS, RESULTS, AND CONCLUSIONS: Transfection experiments using geneticin (G418) selection to identify the specific protein S-thiolated substrates of glutaredoxin-1 (Grx-1) noted the curious phenomenon that non-transfected control cells treated with G418 had increased levels of Grx-1 expression. Varied concentrations of gentamicin, kanamycin, and hygromycin increased Grx-1 expression in a time- and dose-dependent fashion in human cultured retinal pigment epithelial cells. Reactive oxygen species formation after aminoglycoside exposure correlated directly to aminoglycoside treatment. Further indication that oxidation regulates Grx-1 expression was noted by the positive effect of phorbol 12-myristate 13-acetate, a known inducer of redox-sensitive AP-1 transcription factor. In agreement with this hypothesis was the finding that the physiologic reductant N-acetylcysteine decreased Grx-1 expression whereas tert-butyl hydroperoxide increased Grx-1 expression. Our data suggest that aminoglycosides increased Grx-1 expression in response to oxidative stress.
The Effect of Arteriovenous Sheathotomy on Cystoid Macular Oedema Secondary to Branch Retinal Vein Occlusion

M.T. Cahill, Peter K. Kaiser, Jonathan E. Sears, S. Fekrat

PURPOSE: Arteriovenous (AV) sheathotomy, a potential treatment for branch retinal vein occlusion (BVO), surgically separates retinal vessels at an AV crossing. Relief of the aetiological obstruction, with resolution of cystoid macular oedema (CMO), may result in improved visual acuity.

METHODS: A retrospective review of consecutive cases of AV sheathotomy for BVO was undertaken. Eyes were categorised as having resolution (group 1), reduction (group 2), or persistence (group 3) of CMO. Intergroup comparisons were made with regard to preoperative, intraoperative, and postoperative parameters. Preoperative and postoperative visual acuities were compared within each group.

RESULTS: Of the 27 eyes identified, eight (29.6%) had resolution, 14 (51.8%) had reduction, and five (18.6%) had persistence of CMO. Median preoperative visual acuity was similar in all groups (1.0, 1.0, 1.3, respectively; p = 0.29). Overall median follow up was 12.0 months (Q1 = 12.0, Q2 = 22.5). Eyes in group 1 had significantly better median postoperative visual acuity than eyes in groups 2 and 3 (0.6, 1.0, 2.0 respectively; p = 0.01). A significantly higher proportion of eyes in group 1 had visual acuity improvement compared with eyes in the other groups (87.5% v 35.7% and 20.0%; p = 0.03). Median postoperative visual acuity was significantly better than median preoperative visual acuity in group 1 eyes only (p = 0.02). A higher percentage of group 1 eyes had evidence of postoperative retinal perfusion (83.0% v 21.43% and 40.0%; p = 0.16). Postoperative retinal detachment occurred in three eyes (11.1%).

CONCLUSION: Complete resolution of CMO after AV sheathotomy occurred in one third of patients, and postoperative vision improved significantly in this group. However, in the majority of cases, despite an improvement in CMO, there was no improvement in vision after AV sheathotomy.
Effects of Overexpression of Bestrophin and Best Macular Dystrophy Associated Mutants of Bestrophin on the Rat DC-ERG


PURPOSE: To determine the effects of wild type and mutant bestrophin overexpression on the light peak of the rat DC-ERG.

METHODS: Adenovirus-mediated gene transfer was used to express wild type bestrophin (wt-best), or two bestrophin mutants (W93C, R218C) associated with Best macular dystrophy (BMD) in the RPE of adult Long-Evans rats. Two weeks after subretinal injection, the retina and RPE were analyzed by fundus exam, conventional and DC-ERG recordings, histology, and immunofluorescence.

RESULTS: Using doses between 0.2 x 10^7 and 5 x 10^7 pfu, wt-best and both the W93C and R218C mutants correctly localized to the basolateral plasma membrane of the RPE. No defects were noted on fundus exams and the morphology of the retina, RPE, and choroid was typically preserved. While an effect of viral load was noted on conventional ERG amplitudes, these effects did not differ from a null virus control. DC-ERGs recorded in response to a 5-min flash stimulus displayed consistent changes in the light peak (LP) when compared against sham- or null virus-injected controls. While LP amplitude did not change in eyes overexpressing wt-best, the LP time constant was significantly accelerated. In eyes overexpressing W93C or R218C, LP amplitude was significantly lowered vs. null controls. In addition, was significantly slowed in W93C animals but unaltered in animals receiving R218C. In normal, control, or wt-best rats, LP onset occurred ~112 sec after stimulus presentation. In eyes receiving R218C, LP onset was delayed, to 130-135 sec. No defects in LP sensitivity were noted, although the LP continued to increase with longer stimulus durations in W93C transduced eyes only.

CONCLUSIONS: Overexpression of wt-best accelerates the LP kinetics, but does not affect LP amplitude or the other components of the DC-ERG. BMD associated mutants exhibit LP reductions and delays that mimic the electrooculographic abnormalities seen in BMD patients. Differences between the effects induced by W93C and R218C suggest a complex mechanism behind bestrophin activity.
Correlation of Bestrophin Protein Expression in the Basolateral Plasma Membrane of the Mouse RPE with the Onset of Photoreceptor Activity in the Retina

B. Bakall, Neil S. Peachey, L.Y. Marmorstein, C. Wadelius, Alan D. Marmorstein

PURPOSE: Best macular dystrophy is an autosomal dominant disease, caused by mutations in the VMD2 gene that encodes the protein bestrophin. To increase our understanding of the function of bestrophin, the onset of bestrophin expression was followed in mouse eyes during development.

METHODS: BALBc mice were examined at different embryonic (E) and postnatal (P) ages. Bestrophin mRNA was quantified using Taq-man quantitative PCR. Immunohistochemistry for mouse bestrophin was performed on paraffin-embedded sections, using a polyclonal mouse bestrophin antibody produced by immunizing rabbits with a peptide corresponding to the mouse bestrophin C-terminus. Electroretinograms were recorded from the corneal surface of mice beginning at P8, to strobe flashes after overnight dark adaptation.

RESULTS: Bestrophin mRNA was detected as early as E15. Bestrophin protein expression in the RPE was not observed until P10 at which time it was observed to localize to the basolateral membrane of the RPE. The earliest age at which an a-wave could be recorded from mouse eyes was P10, coincident with the onset of bestrophin protein expression in the RPE.

CONCLUSIONS: Despite the presence of mRNA for Bestrophin during embryonic development, the onset of protein expression in RPE is late, beginning at P10. This coincides with the onset of photoreceptor electrical activity, supporting the hypothesis that bestrophin plays a role in the RPE electrical responses to phototransduction. In addition, we conclude that bestrophin protein is a late marker for RPE differentiation.
An Improved Adult Animal Model of Smith-Lemli-Opitz Syndrome


PURPOSE: Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disease caused by a defect in cholesterol (Chol) biosynthesis, at the level of 3β-hydroxysterol-Δ7-reductase. Prior SLOS animal models have employed either feeding or bolus injection of inhibitors of the reductase (AY9944 or BM15.766). To provide a more controlled pharmacological model of SLOS, we tested the efficacy of continuous systemic AY9944 delivery in adult rats, using implanted osmotic pumps.

METHODS: Alzet pumps (Model 2ML4, 28-day) containing AY9944 (2 ml, 50 mg/ml water, 2.5 l/h) were implanted into anesthetized adult (3-mo. old, ca. 250 g) female Sprague-Dawley rats (N=10) under the dorsal skin. Over a 3-mo. Period, new pumps were inserted and old pumps removed every 4 wk. Rats were maintained under dim cyclic light (12L:12D, 20-40 lux) and fed Chol-free chow and water ad lib. Serum sterol levels were monitored by HPLC bi-weekly. After dark- and light-adapted ERGs were recorded, one eye from each rat was taken for histological and quantitative morphometric analysis, while contralateral retinas, livers, and brains, were harvested for sterol analysis. The results were compared against those obtained previously from control rats.

RESULTS: 7-dehydrocholesterol (7DHC) to Chol mole ratio in all tissues of treated rats was >5:1; by contrast, 7DHC/Chol < 0.01 in control tissues. Dark- and light-adapted ERG amplitudes were markedly reduced, and their implicit times were substantially elevated, relative to controls. Outer nuclear layer (ONL) thickness was reduced by 20-30%, relative to controls.

CONCLUSIONS: Osmotic pump delivery of AY9944 offers an improved SLOS animal model, obviating the tedium and inherent variability of daily drug administration by diet or injections, while providing reliable, uniform, and continuous drug delivery at pharmacologically effective levels. Retinal degeneration and functional deficits were even greater than those previously obtained by systemic administration of AY9944 during gestational and postnatal development in rats (Fliesler et al., ARVO 2002), and also are consistent with retinal dysfunction.
Nyctalopin Is Required for Signaling Through Depolarizing Bipolar Cells in the Murine Retina

R.G. Gregg, P.D. Lukasiewicz, Neil S. Peachey, B.T. Sagdullaev, M.A. McCall

PURPOSE: The nob mouse mutant, which lacks nyctalopin, has no b-wave. We investigated whether this defect was attributed to the inability of depolarizing bipolar cells (DBCs) to respond to glutamate. To determine whether glutamate release from photoreceptors was compromised, visual response properties of nob retinal ganglion cells were examined.

METHODS: Whole cell recordings from bipolar cells were made in retinal slices from adult normal and nob mice. Current responses to puffs of glutamate were obtained under voltage clamp conditions. Each bipolar cell was filled with Lucifer yellow and classified by its morphology and the laminar location of its cell body and axon terminals. Ganglion cell recordings were made in vivo from the optic nerve, using tungsten electrodes. Computer driven visual stimuli were used to characterize their response properties.

RESULTS: In retinas from normal mice, glutamate puffs produced a robust outward current in DBCs and an inward current in hyperpolarizing bipolar cells (HBCs). By contrast, in nob retinas, only HBCs responded to glutamate. The inability to respond to glutamate suggests the nob defect resides in the DBCs. In normal mice, visual responses from ON and OFF center ganglion cells were recorded in similar proportions. In nob retinas only OFF center cells responded to light stimulation.

CONCLUSION: These data indicate nyctalopin is required for modulation by glutamate of the cation current in DBCs and visual processing through the ON pathway.
Evaluation of Inner Retinal Structure in the Aged RCS Rat

M. Blum, M.T. Pardue, B. Hanzlicek, Neil S. Peachey, S.L. Ball

PURPOSE: In retinitis pigmentosa (RP), loss of visual function is predominantly due to the loss of rod photoreceptors. A number of labs are currently evaluating whether RP may be treated by replacing the diseased photoreceptors with either healthy cells or a photosensitive prosthetic device. As these efforts are critically dependent upon the maintenance of an intact inner retinal circuitry, we evaluated inner retinal structure and function in a widely used model of photoreceptor degeneration, the RCS rat.

METHODS: Eyes were collected from deeply anesthetized pigmented dystrophic RCS rats and controls from 3 weeks to 12 months of age, immersion fixed in 4% paraformaldehyde for 30 min, cryoprotected, embedded and sectioned at 10 µm. Immunohistochemistry was completed using standard protocols with the following antibodies: PKC or recoverin, to label rod and cone bipolar cells, respectively, and calretinin, parvalbumin, choline acetyltransferase, or tyrosine hydroxylase to label subclasses of amacrine cells.

RESULTS: In addition to observing a retraction of rod bipolar cell projections similar to that previously described (Hanitzsch et al., 1998), we observed an increase in the intensity of recoverin label in cone bipolar cell somas. We found a normal laminar labeling pattern for all amacrine cell markers in the RCS inner nuclear and plexiform layers.

CONCLUSION: This study identified changes in the cone visual pathway which may be important in considering treatment strategies for RP. In comparison, amacrine cell organization appears to be well maintained in the RCS rat during photoreceptor degeneration.
Immunohistochemical Analysis of the Outer Plexiform Layer in the Nob Mouse

S.L. Ball, M.T. Pardue, M.A. McCall, R.G. Gregg, Neil S. Peachey

PURPOSE: In nob (no-b-wave) mice, a mutation in the gene that encodes for the nycalopin protein results in a defect in communication between photoreceptors and depolarizing bipolar cells (DBCs). As the role of the nycalopin protein is unknown, we investigated possible effects of this mutation on the distribution of proteins that are necessary for DBC activation or the generation of a b-wave.

METHODS: Adult normal and nob mice were enucleated, and the eyes were immersion fixed in 4% paraformaldehyde for 10 min, cryoprotected, embedded and sectioned on a cryostat at 10 µm. Immunohistochemistry reactions were performed using standard protocols with antibodies to the following proteins: PKC, PSD-95, the α1F subunit of voltage gated calcium channels, mGluR6, G0α, bassoon, trkB, and dystrophin.

RESULTS: In control retinas, each antibody showed a labeling pattern in the OPL that was comparable to those previously described for mouse retina. In nob mice, the labeling pattern was comparable to controls.

CONCLUSIONS: The normal distribution of these OPL synaptic proteins in nob mice leads us to two possible explanations. First, although these proteins are correctly localized, in the absence of nycalopin one or more may not be functional. Alternatively, the defect associated with nob may involve a novel role for nycalopin in synaptic transmission or the DBC signal transduction cascade.
Pharmacological Analysis of the Rat Cone Electroretinogram

L. Xu, S.L. Ball, K.R. Alexander, Neil S. Peachey

PURPOSE: The electroretinogram (ERG) of the cone system provides a useful noninvasive measure of the activity of the cone pathway. Despite a wide application of the cone ERG in the study of rodent models of human hereditary retinal disease, the cellular origins of the rat cone ERG have not been well defined. Here, we address this issue using a pharmacological approach that has been used previously to derive ERG response components.

METHODS: Agents that impair synaptic transmission at well-defined retinal loci were dissolved in saline and injected into the vitreous of adult Sprague-Dawley rats anesthetized with ketamine/xylazine, and cone ERGs were recorded approximately 2 h later.

RESULTS: Analysis of the resulting waveforms indicated that the rat cone ERG includes a relatively small-amplitude component of negative polarity that is derived from the activity of cone photoreceptors, and perhaps retinal glial (Muller) cells. The cone depolarizing bipolar cell pathway contributes a positive potential of large amplitude to the rat cone ERG. In comparison, the contribution of hyperpolarizing bipolar cells is of negative polarity and of much smaller amplitude. The inner retina contributes a negative wave upon which higher frequency oscillations are superimposed.

CONCLUSIONS: These results provide a foundation for interpreting changes in the waveform of the rat cone ERG that may be observed following genetic alteration or other experimental treatment.
Electrophysiological Analysis of Visual Function in Mutant Mice
Neil S. Peachey, S.L. Ball

PURPOSE: The mouse has become a key animal model for ocular research. This situation reflects the fact that genes implicated in human retinal disorders or in mammalian retinal function may be readily manipulated in the mouse. Visual electrophysiology provides a means to examine retinal function in mutant mice, and stimulation and recording protocols have been developed that allow the activity of many classes of retinal neurons to be examined and which take into account unique features of the mouse retina.

METHODS: Here, we review the mouse visual electrophysiology literature, covering techniques used to record the mouse electroretinogram and visual evoked potential, and how these have been applied to characterize the functional implications of gene mutation or manipulation in the mouse retina.
In Vivo Gene Transfer as a Means to Study the Physiology and Morphogenesis of the Retinal Pigment Epithelium in the Rat

Alan D. Marmorstein, Neil S. Peachey, K.G. Csaky

PURPOSE: Our understanding of the morphogenesis of epithelial phenotypes has been greatly advanced by the use of in vitro cell culture systems. However, cell cultures often do not faithfully reconstitute many of the differentiated properties of the cell from which they are derived and cannot be used to examine complex physiologic interactions between adjacent tissues. This is particularly true of the retinal pigment epithelium (RPE). Many plasma membrane proteins, in vivo, exhibit a reversed polarity with respect to other epithelia, and RPE-derived cell lines seldom exhibit these same polarity properties. Furthermore, the interaction between the RPE cell and the neurosensory retina, or the underlying blood supply, the choroid, is absent in cell culture. Most epithelia are difficult to isolate and study in vivo. The RPE is an exception to this.

METHODS: We have explored several aspects of RPE protein transport properties, vision-related physiology, and disease-related pathophysiology in the eye using in vivo gene transfer and electrophysiologic techniques. By injecting replication-defective adenoviruses into the subretinal space of rat eyes, we have been able to easily direct the expression of a test protein and follow its sorting and physiologic effects on RPE cells and adjacent tissues.

RESULTS AND CONCLUSIONS: Due to binding and internalization of adenoviral vectors to integrins found on the RPE apical plasma membrane, expression in a healthy eye is essentially confined to the RPE cell, even under control of a cytomegalovirus promotor. The use of varying amounts of adenoviral vector allows for determination of dose-responsive effects and the comparison of multiple mutants of a protein. In addition, there are substantial savings with respect to time and money in comparison to standard transgenic approaches.
PARP Inhibitor Corrects Diabetes-Induced Alterations in Retinal Function and Leukostasis


PURPOSE: PARP ((poly(ADP-ribose) polymerase-1) is a nuclear DNA nick-sensor enzyme involved in the poly(ADP-ribosylation). Potential adverse effects of PARP overactivation include damaging cells via energy depletion. We have assessed the effect of a potent PARP inhibitor (PJ34) on diabetes-induced abnormalities of retinal function (ERG), ICAM expression and leukostasis within retinal vessels, and on “hyperglycemia”-induced death of retinal endothelial cells and pericytes (assessed with trypan blue) in vitro.

METHODS: Experimentally diabetic rats (2 weeks to 2 months) were assigned to PJ34 (20mg/kg BW) or control groups. Bovine retinal endothelial cells were incubated in 5 and 25 mM glucose for 5 days with and without PJ34. Leukostasis was determined in vivo by counting fluorescent leukocytes remaining in the retinal vasculature after perfusing the animal with conconavalin A-FITC and then buffer.

RESULTS AND CONCLUSIONS: Elevated glucose caused a significant increase in death of both capillary cell types in vitro, and this was inhibited in a dose-dependent manner by PJ34. In vivo, diabetes increased activity of PARP in retina and retinal capillary endothelial cells and pericytes. Diabetes also resulted in increased expression of retinal ICAM, abnormal ERG, and leukostasis within retinal vessels. PJ34 significantly inhibited all of these defects. Inhibition of PARP corrects several metabolic and physiologic defects associated with the development of diabetic retinopathy. Long-term trials to assess the role of PARP in the development of morphologic alterations characteristic of diabetic retinopathy are underway.
Oxidative Protein Modifications and Age-Related Vision Loss

John W. Crabb, Xiaorong Gu, Kutralanathan Renganathan, Masaru Miyagi, Quteba Ebrahem, Bela Anand-Apte, Daniel T. Organisciak, Robert G. Salomon, Joe G. Hollyfield

PURPOSE: Age-related macular degeneration (AMD) is the most common cause of legal blindness in the elderly population of developed countries. Both genetic and environmental factors contribute to the disease however the cause of AMD is unknown and presently there are no cures. We hypothesize that similar mechanisms of oxidative damage are involved in AMD and retinal light damage and are identifying proteins and protein chemical modifications associated with AMD and light damaged rat retina as an approach to defining these pathways. Major risk factors for developing AMD are extracellular deposits termed drusen, which accumulate with age beneath the retinal pigment epithelium on Bruch’s membrane. The progression of AMD might be slowed or halted if the formation of drusen could be modulated.

METHODS AND RESULTS: Liquid chromatography tandem mass spectrometric (LC MS/MS) analyses of drusen preparations from 18 normal human donors and 5 AMD donors identified 129 proteins [2002 Proc Natl Acad Sci USA 99, 14682]. Immunocytochemical studies have thus far localized about 16% of these proteins in drusen. TIMP3, clusterin, vitronectin, and serum albumin were the most common proteins observed in normal donor drusen while crystallin was detected more frequently in AMD drusen. Interestingly, crystallins also appear to be more abundant in rat retina following light damage [2003 Exp Eye Res 76, 131]. Oxidative protein modifications identified in AMD tissues include apparent crosslinked species of TIMP3 and vitronectin, carboxymethyl lysine and carboxyethyl pyrrole (CEP) protein adducts. CEP adducts are uniquely generated from the oxidation of docosahexaenoate-containing lipids and by Western analysis, are more abundant in AMD than in normal Bruch’s membrane. Late stage AMD, with the most severe vision loss, involves neovascularization where blood vessels grow from the underlying choroid through Bruch’s membrane into the retina. Our recent results demonstrate that CEP adducts stimulate angiogenesis in vivo in chorioallantoic membrane and corneal implant assays. CEP immunoreactivity and CEP autoantibody titer are also significantly elevated in plasma from AMD donors relative to that from age-matched normal donors, and may be of diagnostic utility as biomarkers for predicting AMD susceptibility. Oxidative protein modifications identified in rat retina following intense in vivo light exposure include CEP adducts, argpyrimidine and nitrotyrosine [2002 Mol Cell Proteomics 1, 293].

CONCLUSIONS: These data link oxidative injury with AMD and retinal light damage and support a role for oxidative protein modifications in their respective mechanisms of pathogenesis.
Characterization of Lipid Oxidation Products and Proteins in Bruch’s Membrane from Normal and AMD Donor Eyes

Xiarong Gu, Karen Shadrach, M Sun, Karen A. West, SL Hazan, RG Salomon, Joe G. Hollyfield, John W. Crabb

PURPOSE: Bruch’s membrane, located beneath the retina, thickens and losses permeability in age-related macular degeneration (AMD). We are probing the possible role of lipid peroxidation and protein modification in this pathology.

METHODS: Bruch’s membrane was isolated free of the adjacent tissues from 5 normal and 4 AMD donor eyes and in the presence of antioxidants. Lipids were extracted with chloroform/methanol and analyzed by LC MS. Proteins were subjected to 1D SDS-PAGE, and either blotted to PVDF membrane or gel bands were excised and proteins identified by LC MS/MS. Western analyses were used to screen for oxidative protein modifications.

RESULTS: Lipid oxidation products from docosahexaenoyl phosphatidylcholine (DHA-PC), arachidonoyl (AA)-PC, and linoleyl (LA)-PC were detected in relatively greater amounts from AMD than age matched healthy donor eyes. Many of the proteins we recently identified in drusen (2002 PNAS 23, 14682-14687) were also detected in Bruch’s membrane, including tissue inhibitor metalloproteinase-3, clusterin, vitronectin and serum albumin. By Western analysis, carboxyethyl pyrrole protein adducts, generated from the oxidation of DHA containing lipids, were more abundant in Bruch’s membrane from AMD than normal donors. Apparent crosslinks were also detected in Bruch’s membrane proteins.

CONCLUSIONS: These preliminary observations support a role for oxidative damage in the pathology of AMD. Efforts are focused upon developing methods for characterizing low abundancy lipid oxidation products and oxidative protein modifications in ocular tissues.
Human Retinal Pigment Epithelium Protein Database

Karen A. West, J Sun, L Yan, Karen Shadrach, A Hasan, M Miyagi, Jack S. Crabb, Joe G. Hollyfield, Alan D. Marmorstein, John W. Crabb

PURPOSE: The retinal pigment epithelium (RPE) is a single cell layer that separates the photoreceptor cells of the retina from their principal blood supply in the choroid. In all vertebrates, the RPE is responsible for vectorial transport of nutrients to rod and cone photoreceptors, removal of waste products to the blood, absorption of scattered light and regeneration of bleached visual pigment. To facilitate studies of RPE physiology, we have initiated the development of a human RPE protein database.

METHODS: RPE cells were isolated from normal adult human donor eyes, subcellular fractions prepared and proteins fractionated by 1D and 2D electrophoresis. Following in-gel proteolysis, tryptic digests were analyzed by LC MS/MS peptide sequencing and/or MALDI TOF peptide mass mapping and the data used to query protein sequence databases.

RESULTS: Preliminary analyses have identified 278 proteins and provide a starting point for building a database of the human RPE proteome. The 1D and 2D PAGE methods were complimentary, each contributing unique protein identifications. One hundred sixty (160) proteins were identified following 2D PAGE, 180 proteins following 1D PAGE, and 62 proteins were identified from both 1D and 2D gels. Ten of the 278 identified proteins are currently designated hypothetical or of unknown function and ~6% were identified based on homology with other species.

CONCLUSIONS: A number of proteins were identified that are known to be associated with specialized functions of the RPE. These included proteins associated with retinoid metabolism and the visual cycle such as cellular retinaldehyde-binding protein, cellular retinol-binding protein, retinal pigment epithelium 65, 11-cis-retinol dehydrogenase 5, retinal G protein-coupled receptor and interphotoreceptor-retinoid binding protein (IRBP). The RPE is the most active phagocytic tissue in humans. Identified proteins involved in macromolecular degradation included cathepsins B, D and Z, lysozyme and several proteasome components. The photooxidative environment in the retina and active phagocytic processing provide abundant reactive oxygen species to the RPE. Identified anti-oxidant proteins included thioredoxin dependent peroxide reductase 1 and 2, catalase, peroxiredoxin 6, superoxide dismutase, glutathione S-transferase and thioredoxin peroxidase.
Biomarkers for Age-Related Macular Degeneration
Jiayin Gu, Xiaorong Gu, Joe G. Hollyfield, Robert G. Salomon, John W. Crabb

PURPOSE: Age-related macular degeneration (AMD) is a slow, progressive disease with genetic and environmental risk factors. Free radical-induced oxidation of docosahexaenoate (DHA)-containing lipids generates carboxyethylpyrrole (CEP) protein adducts that are more abundant in ocular tissues from AMD than normal human donors. To probe for possible biomarkers of AMD susceptibility, we have initiated analysis of CEP immunoreactivity and autoantibodies in human plasma.

METHODS: Blood was collected from AMD and normal, healthy donors at the Cole Eye Institute, Cleveland Clinic Foundation and Massachusetts Eye and Ear Infirmary, Boston, MA. Plasma CEP immunoreactivity and CEP autoantibody titer were determined by ELISA using rabbit polyclonal anti-CEP antibody and CEP modified albumin as a reference standard.

RESULTS: The mean level of anti-CEP immunoreactivity in AMD plasma (n = 19 donors) was 1.5-fold higher (p = 0.004) than in age-matched controls (n = 19 donors). Sera from AMD patients demonstrated mean titers of anti-CEP autoantibody 2.3-fold higher than controls (p = 0.02). Of individuals (n = 13) exhibiting both antigen and autoantibody levels above the mean for non-AMD controls, 92% had AMD. Logistic regression modeling of these data support a higher probability of correctly predicting AMD using both CEP immunoreactivity and CEP autoantibody titer than using either variable alone.

CONCLUSIONS: A combination of plasma CEP immunoreactivity and autoantibody titer may have diagnostic utility in identifying those susceptible of developing AMD before the manifestation of retinal pathology. A much larger clinical investigation is now underway to test whether this approach could be useful in predicting AMD susceptibility.
Mechanism of Induction of Choroidal Neovascularization in Sorsby Fundus Dystrophy

Bela Anand-Apte, Jia Hua Qi, Quteba Ebrahem, G. Murphy, L. Claesson

PURPOSE: Sorsby fundus dystrophy (SFD) is a dominantly inherited condition characterized by the development of choroidal neovascularization, subretinal hemorrhages and changes consistent with disciform degeneration. Mutations in the Tissue Inhibitor of Metalloproteinases-3 (TIMP-3) gene, all of which introduce an extra cysteine residue into exon 5, cause Sorsby Fundus Dystrophy (SFD). TIMP-3, a regulator of matrix metalloproteinases (MMPs) is deposited by retinal pigment epithelial (RPE) cells into Bruch’s membrane (BM) where it is a component of the extracellular matrix. In this study we set out to elucidate the mechanism by which TIMP-3 mutations induce the angiogenic phenotype.

METHODS: We have introduced TIMP-3 into porcine aortic endothelial cells expressing VEGFR-2 as well as human retinal pigment epithelial cells. In vitro, VEGF induced proliferation and migration of endothelial cells were analyzed by Coulter particle counting and modified Boyden chamber assays respectively. VEGF induced autophosphorylation of VEGFR-2 and activation of p44/p42 MAP kinase was evaluated by western blot analysis. Conditioned medium from RPE cells was tested for their ability to induce angiogenesis in an in vivo chick chorio-allantoic membrane (CAM) assay.

RESULTS: TIMP-3 over-expression in endothelial cells resulted in an inhibition of the proliferative and migratory response of these cells to VEGF. VEGF-induced autophosphorylation of VEGFR-2 and p44/p42 MAP kinase activation were also suppressed. These effects appear to be independent of its MMP inhibitory activity. Conditioned medium from RPE cells expressing SFD mutant TIMP-3 demonstrated increased MMP activity as well as increased angiogenic activity on CAM assay.

CONCLUSIONS: Our data indicate that expression of SFD mutant TIMP-3 in RPE cells results in increased MMP activity and induction of angiogenesis.
Induction of Angiogenesis by Active Matrix Metalloproteinases-2 and 9: Role of VEGF

Quteba Ebrahem, Bela Anand-Apte

PURPOSE: Matrix metalloproteinases (MMPs) are a specialized group of enzymes that participate in extracellular matrix (ECM) degradation and have been postulated to play an important role in angiogenesis. The purpose of this study was to determine if active MMPs could induce angiogenesis in vivo.

METHODS: The chick chorio-allantoic membrane (CAM) assay was used as an in vivo angiogenesis model in this study. Methylcellulose discs containing pro or active MMPs were placed on 6 day CAMs and analyzed for their ability to induce a neovascularization response surrounding the disc after 48 hours. The ability of a neutralizing VEGF antibody to inhibit this response was examined.

RESULTS: Active MMPs can initiate an angiogenic response in the CAM assay in the form of increasing tortuosity of vessels surrounding the disc. This effect was not observed with pro MMPs. Neutralizing antibodies to VEGF can inhibit this response. In addition, synthetic inhibitor of MMPs can inhibit the angiogenic response of VEGF.

CONCLUSIONS: Active MMPs can initiate an angiogenic response by increasing the tortuosity of capillaries mediated by release of low levels of VEGF. Our data suggests that MMPs may act upstream as well as downstream of VEGF during in vivo angiogenesis.
ADAMTSL-3/Punctin-2, a Novel Glycoprotein in Extracellular Matrix Related to the ADAMTS Family of Metalloproteases

Nina Hall, Phil Klenotic, Bela Anand-Apte, S.S. Apte

PURPOSE: To identify novel proteins related to the ADAMTS family of metalloproteases.

METHODS: The complete primary structure of ADAMTSL-3/punctin-2, a novel member of the family designated ADAMTSL (a disintegrin-like and metalloprotease domain with thrombospondin type I motifs-like), was determined by cDNA cloning from a human placenta library.

RESULTS: The predicted open reading frame encodes a protein of 1690 amino acids that has considerable similarity to ADAMTSL-1/punctin-1. These multi-domain proteins lack both a protease domain and a disintegrin-like domain but are remarkably similar in their domain organization to the ADAMTS proteases, hence the name ADAMTS-like. Punctin-2 contains thrombospondin type 1 repeats (TSRs), a cysteine-rich domain and a cysteine-free spacer domain in the precise order in which they occur in the ADAMTS proteases. However, the number and organization of the TSRs in punctin-2 is unique with respect to the ADAMTS proteases. Punctin-2 contains 13 TSRs arranged in two arrays separated by a region containing three immunoglobulin-like repeats. Northern blot analysis of RNA from human adult tissues demonstrated that ADAMTSL3 is widely expressed, with highest expression in liver, kidney, heart and skeletal muscle, whereas it is expressed at low levels in mouse embryos. We characterized two punctin-2 polyclonal antisera. Using these and a monoclonal antibody to a C-terminal myc tag, we show that in transfected COS-7 cells, punctin-2 is expressed as a 210-kDa glycoprotein that is located in the extracellular matrix.

CONCLUSIONS: The domain structure of punctin-2 and its matrix localization suggest that it might play a role in cell-matrix interactions or in assembly of specific extracellular matrices.
Differential Caveolin-1 Polarization in Endothelial Cells During Migration in Two and Three Dimensions

Marie-Odile Parat, Bela Anand-Apte, Paul L. Fox

PURPOSE: Endothelial cell (EC) migration is a critical event during multiple physiological and pathological processes. ECs move in the plane of the endothelium to heal superficially injured blood vessels but migrate in three dimensions during angiogenesis. We herein investigate differences in these modes of movement focusing on caveolae and their defining protein caveolin-1.

METHODS: Using a novel approach for morphological analysis of transmigrating cells, we show that ECs exhibit a polarized distribution of caveolin-1 when traversing a filter pore.

RESULTS: Strikingly, in these cells caveolin-1 seems to be released from caveolar structures in the cell rear and to relocalize at the cell front in a cytoplasmic form. In contrast, during planar movement caveolin-1 is concentrated at the rear of ECs, colocalizing with caveolae. The phosphorylatable Tyr14 residue of caveolin-1 is required for polarization of the protein during transmigration but does not alter polarization during planar movement. Palmitoylation of caveolin-1 is not essential for redistribution of the protein during either mode of movement.

CONCLUSIONS: Thus, ECs migrating in three dimensions uniquely exhibit dissociation of caveolin-1 from caveolae and phosphorylation-dependent relocalization to the cell front.
A Novel Function for Tissue Inhibitor of Metalloproteinases-3 (TIMP3): Inhibition of Angiogenesis by Blockage of VEGF Binding to VEGF Receptor-2

Jian-Hua Qi, Quteba Ebrahem, N. Moore, G. Murphy, L. Claesson-Welsh, M. Bond, A. Baker, Bela Anand-Apte

BACKGROUND: Tissue inhibitor of metalloproteinases-3 (TIMP3) is one of four members of a family of proteins that were originally classified according to their ability to inhibit matrix metalloproteinases (MMP). TIMP3, which encodes a potent angiogenesis inhibitor, is mutated in Sorsby fundus dystrophy, a macular degenerative disease with submacular choroidal neovascularization. In this study we demonstrate the ability of TIMP3 to inhibit vascular endothelial factor (VEGF)-mediated angiogenesis and identify the potential mechanism by which this occurs.

CONCLUSIONS: TIMP3 blocks the binding of VEGF to VEGF receptor-2 and inhibits downstream signaling and angiogenesis. This property seems to be independent of its MMP-inhibitory activity, indicating a new function for this molecule.
Section 10: Uveitis
High-Speed Optical Coherence Tomography of Anterior Segment Surgical Anatomy and Pathology

David Huang, Maria Regina Chalita, Y. Li, Careen Y. Lowder, David M. Meisler, A.M. Rollins, J.A. Izatt

PURPOSE: To use a high-speed corneal and anterior segment optical coherence tomography (CAS-OCT) system to image ocular pathologies and surgical anatomy.

METHODS: A high-speed (4000 a-scan/sec) wide-field (16 mm) CAS-OCT system was developed. It uses a longer wavelength (1.3 microns) compared to retinal OCT systems (0.8 microns). OCT scans were performed on 11 eyes with anatomic features of interest.

RESULTS: OCT of post-surgical cornea (LASIK, penetrating keratoplasty), trabeculectomy bleb, anterior chamber intraocular lens (IOL), iris masses and cataract were obtained. Full-thickness imaging of sclera, angle and iris was possible. No appreciable motion artifact was noted at 8 frames/sec. The entire LASIK flap could be fully visualized. In keratectasia, OCT showed relative corneal thinning in the area of steepening. Causative factor such as inadequate residual posterior stromal thickness and excessive flap thickness could be quantitatively assessed. The longer 1.3-micron wavelength allowed the angle recesses to be visualized. The recess-to-recess anterior chamber width could be directly measured, along with other parameters such as the anterior chamber depth and crystalline lens vault. In trabeculectomy images, the sclerotomy site could be visualized as well as the whole bleb anatomy. The anterior chamber IOL as seen and the footplates position were recorded. Iris and ciliary body masses could be precisely delineated and accurately measured.

CONCLUSIONS: The CAS-OCT prototype allowed non-contact visualization and measurement of corneal and anterior segment pathologies and surgical anatomy. The high speed allows quantitative measurements of relevant biometric dimensions. The longer wavelength (1.3-micron) allows greater penetration through highly scattering tissue such as limbus and sclera.
Ultrasound Biomicroscopy (UBM) of Ciliary Processes in Uveitis

Egbert Saavedra, D. Socci da Costa, Luanne Sculley, Careen Y. Lowder

PURPOSE: To determine the effect of duration, severity, and location of uveitis on the length of ciliary processes.

METHODS: Ultrasound biomicroscopy (UBM) of ciliary processes was obtained in superior, temporal, nasal, and inferior quadrants in 91 uveitic eyes and 16 normal eyes. The five longest ciliary processes measured in microns from each quadrant were analyzed with regards to duration (acute, chronic, and recurrent), severity (aggressive, moderate, and mild), and location (anterior, intermediate, posterior, and diffuse) of uveitis.

RESULTS: Ciliary processes measurements were as follows. Duration: inferior (chronic, 356.33; acute, 423.96; normal, 534.64; P = 0.004); nasal (chronic, 434.69; acute, 457.46; normal, 565.33; P = 0.017); temporal (chronic, 467.75; acute, 487.75; normal, 582.48; P = 0.015); and superior (chronic, 498.70; acute, 549.97; normal, 581.17; P = 0.127). Severity: inferior (aggressive, 334.38; moderate, 392.59; mild, 429.50; normal, 523.23; P = 0.007); nasal (aggressive, 413.79; moderate, 465.99; mild, 444.98; normal, 562.74; P = 0.018); temporal (aggressive, 429.52; moderate, 509.47; Mild, 492.55; normal, 577.48; P = 0.007); and superior (aggressive, 480.44; moderate, 558.62; mild, 480.08; normal, 568.33; P = 0.146). Location: inferior (diffuse, 338.57; intermediate, 533.00; anterior, 421.18; normal, 530.81; P = 0.003), nasal (diffuse, 423.04; intermediate, 502.90; anterior, 464.67; normal, 565.47; P = 0.024), temporal (diffuse, 441.29; intermediate, 558.00; anterior, 516.77; normal, 584.99; P = 0.007), and superior (diffuse, 489.25; intermediate, 561.60; anterior, 545.46; normal, 578.05; P = 0.190).

CONCLUSIONS: Significant differences in ciliary process lengths were found between eyes with and without uveitis. Greatest damage to ciliary processes were found in eyes with chronic, aggressive, and diffuse uveitis. Superior quadrant ciliary processes were least susceptible to damage. Information on ciliary processes may be used to guide management of patients with uveitis.
Surgical Management of Cataracts in Children with Juvenile Rheumatoid Arthritis-Associated Uveitis

Linda A. Lam, Careen Y. Lowder, George Baerveldt, Scott D. Smith, Elias I. Traboulsi

PURPOSE: To evaluate outcomes of cataract surgery with posterior chamber intraocular lens (IOL) implantation with or without trabeculectomy in children with juvenile rheumatoid arthritis (JRA)-associated uveitis.

METHODS: Interventional case series; Retrospective chart review of five patients aged 12 years or younger with JRA-associated uveitis who underwent cataract surgery with posterior chamber IOL with or without trabeculectomy at the Cleveland Clinic Foundation from December 1995 to October 2001.

RESULTS: Four female patients and one male patient ranging from age 7 to 12 years were identified. One patient had bilateral involvement; six eyes were included in the study. Three eyes underwent cataract extraction with posterior chamber IOL, and three underwent combined cataract surgery with posterior chamber IOL and trabeculectomy. Median age at surgery was 8.5 years, with a median follow-up of 43.5 months. Four of five children (five eyes) were on systemic methotrexate immunosuppressive therapy for a median length of 1.25 years before surgery. Two of five patients (three eyes) were also on additional systemic immunosuppressive or anti-inflammatory treatments. All eyes received frequent topical corticosteroid therapy for a median of 2 weeks preoperatively and 8.5 weeks postoperatively. A final postoperative Snellen visual acuity of 20/40 or better was achieved in all children. A median final visual acuity improvement of 7 Snellen lines was observed after cataract surgery.

CONCLUSIONS: With adequate long-term preoperative and postoperative control of intraocular inflammation with systemic immunosuppressive therapy in addition to intensive topical corticosteroid treatment, children with JRA-associated uveitis can demonstrate favorable surgical outcomes after cataract surgery with posterior chamber IOL.
Chest Computerized Tomography in the Evaluation of Uveitis

S. Chang, Careen Y. Lowder, Peter K. Kaiser, M.A. Meziane, Thomas W. Rice, David M. Meisler

PURPOSE: Computerized tomography (CT) of the chest was valuable in the evaluation of elderly women with uveitis. We further examined the utility of chest CT in patients with intraocular inflammation of undetermined etiology regardless of age and gender.

METHODS: Prospective case series. Chest CT was performed on all patients with intraocular inflammation without definitive cause between January 2002 and December 2002.

RESULTS: 42 patients (14 males, 28 females), ages 13–88 (median, 54.5 yrs) were included. All patients underwent a battery of diagnostic laboratory studies and chest CT. Chest CT in 16 of 42 (38%) patients was positive for parenchymal, mediastinal, and/or hilar adenopathy. Eight of 16 (50%) were women (41–88 yrs; median, 61.5 yrs) and 8 (50%) were men (26–79 yrs; median 51 yrs). Eleven of 16 patients with positive CT underwent mediastinoscopy. Non-necrotizing granulomas were present in 8 patients (6 females, 2 males), necrotizing granuloma (2), lymph node (1). In additional 6 patients, chest CT revealed breast mass (1), postinflammatory change (1), interstitial fibrosis (1), axillary lymphadenopathy (1), pleural mass (1) and subcutaneous nodule (1).

CONCLUSION: Chest CT is useful in the evaluation of uveitis.
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