1179 - D1111
Time-Dependent Change in Glutathione Peroxidase Activity in H2O2-Treated Lenses From Wild-Type and Thioredoxinase/glutaredoxin-1 Knockout Mice

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Purpose: To study oxidative stress response in cultured lenses.

Methods: Lenses from wild-type and thioredoxinase/glutaredoxin-1 knockout mice were incubated with 0.5 mM H2O2. The lenses were homogenized at day 1, 2, and 4 and the activity of glutathione-related enzymes were quantified photometrically. Protein concentration was quantified using a BCA assay.

Results: The activity of glutathione peroxidase decreased significantly the first two days, and normalized at day 4, with indication of increased activity in the wild-type lenses. The activity at day 1 and 2 was significantly lower than that of day 4. There was no significant difference between the wild-type and the knockout lenses. Other planned enzyme measurements were not finished at the time of the abstract submission.

Conclusions: Glutathione peroxidase activity decreases initially after oxidative stress and recovers later on. This pattern varies with species, tissue and oxidant.

CR: S. Lojen, None; P. Andertun, None.
Support: Wibergs stiftelse, Kronprinsessen Margareta fond, Stiftelsen Synfriamjandets forskningsfond

1180 - D1112
Differences in Ascorbate Distribution and Oxygen Consumption Between the Eyes of Humans and Other Animals

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Purpose: Exposure of the lens to elevated oxygen can cause nuclear cataracts. Our previous study found that the ascorbic acid (AsA) in human vitreous humor reacts with oxygen, presumably lowering oxygen around the lens. We compared the AsA concentration in aqueous and vitreous humor and the rate of oxygen consumption in vitreous from human, cow, pig and rabbit eyes.

Methods: Samples of aqueous and vitreous were obtained from rabbit, pig, cow eyes immediately postmortem and from human eyes at the time of vitrectomy, cataract or glaucoma surgery. AsA was measured colorimetrically in triplicate samples. Oxygen consumption was measured using a microrespirometer fitted with an Oxylab pO21 optical oxygen sensor.

Results: AsA levels in aqueous and vitreous have been measured previously, but usually not from the same eyes and in different species at the same time. Humans had the highest AsA concentration in the vitreous and aqueous (Table). In humans, AsA level was consistently higher in the vitreous than in the aqueous, whereas in the three animal species this ratio was reversed. The average rate of oxygen consumption (μl/ml/hr) was also highest in human vitreous (rabbit 0.99 ± 0.17, pig 0.49 ± 0.18, n=9, cow 0.90 ± 0.15, n=8, human, 1.32 ± 0.13, n=27).

Conclusions: The high AsA levels in human eye suggests that the ocular environment protects the human lens from exposure to oxygen. AsA is believed to enter the eye in the aqueous humor by transport across the ciliary epithelium. Our measurements raise the possibility that an additional transporter pumps AsA into human vitreous, accounting for the higher AsA concentration in the vitreous than in the aqueous. Experiments are in progress to test this possibility in human patients. Differences between humans and animal models must be accounted for in studies of ocular physiology.

CR: F. Bai, None; P. Lei, None; Y.-B. Shui, None; R. Gupta, None; N. Holekamp, None; C. Siegfried, None; D. Beebe, None.
Support: NIH Grant EY015863

1181 - D1113
An Investigation of the Effect of Cataract Surgery and Vitrectomy on the Antioxidant Status of the Aqueous Humour and Vitreous Through the Quantification of Glutathione

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Purpose: The cumulative effects of oxidative stress in the eye over a lifetime can lead to cataract formation and age-related macular degeneration. Glutathione is an important natural free-radical scavenger in the eye. The goal of the study is to use the ratio of oxidized/total glutathione as a marker for oxidative stress and quantify its presence within the aqueous humor and vitreous of patients with different ocular status such as previous cataract surgery and vitrectomy.

Methods: Aqueous humor and vitreous samples from sequential subjects set to undergo pre-scheduled eye surgery were collected upon the initiation of the surgical procedure. They were analyzed using reverse-phase high-performance liquid chromatography (RP-HPLC) for the quantitative presence of glutathione.

Results: There were 56 eyes of 56 patients (31 male and 25 female) in this prospective study. The average age was 68 years. Thirty-six eyes had no previous surgery. Eight eyes had undergone cataract surgery; 5 eyes had vitrectomy and 7 had a combination of the two. Comparing aqueous samples of oxidized glutathione (GSSG)/total glutathione in eyes with no previous surgery and in vitrectomized eyes, it was 15% and 4% respectively (p<0.02). In 12 cases, samples were taken concurrently from both the aqueous humor (AC) and the vitreous (VH). In these patients, the percentage of GSSG to total glutathione was 43% in VH vs. 19% in AC (p=0.01). The axial length measurement of oxidized glutathione ratio. In those with previous surgery compared to vitrectomized eyes. Both findings are surprising given the previous data from oxygen sensor measurements. Possible mechanisms for these findings will be discussed.

CR: C.X. Qian, None; W.S. Powell, None; M. Khuthaila, None; T. Jayasundera, None; S. Gravel, None; J.C. Chen, None.
Support: None

1182 - D1114
Expression and Localization of a Novel Lens Reducing System Called Thioredoxin-Like Protein 6 (TXNL6) in Human Lens and Retina


Purpose: TXNL6 is a thioredoxin-like protein that has been reported to exist as two alternatively spliced forms. The short form, known as Rdcvf (rod-derived cone viability factor), has been implicated in being involved in cone viability and cone survival whereas the long form, TXNL6, is believed to have oxidoreductase activity and function similarly to thioredoxin (TXR) using the CXXC motif. Here, we examined the expression of TXNL6 in human lens and retinal tissues paying particular attention to its subcellular localization in these cells.

Methods: To establish the expression of TXNL6 in human lens and retina, we performed semi-quantitative RT-PCR from RNA isolated from tissues of human lens epithelia, human lens fiber, and human retina. We also examined the protein levels of TXNL6 using extracts isolated from human lens epithelia, human lens fibers, and monkey retina by western blot. We further explored the localization of TXNL6 in HLE and RPE cells by immunofluorescence staining visualized by confocal microscopy. All immunostaining experiments have been performed in two HLE cell lines (SRA01/04 and HLEB3) and in two RPE cell lines (D407 and ARPE19).

Results: Our results demonstrated the expression of TXNL6 in human lens and human/monkey retina at both the transcript and protein levels. Immunostaining of TXNL6 revealed co-localization to the mitochondria as well as throughout the cytosol of HLE and RPE cells. Immunofluorescence data also revealed co-localization of TXNL6 with the repair enzyme, MsrA, in HLE and RPE cells.

Conclusions: Our data show that TXNL6 is expressed in the human lens and retina, is co-localized to the mitochondria, and is co-localized with MsrA. Our results suggest that TXNL6 has an important reducing function in the lens and retina that likely involves serving as a reducing system for MsrA and other mitochondrial protective and repair enzymes.

CR: W. Lee, None; M. Demos, None; R. McGreal, None; L. Brennan, None; M. Kantorow, None.
Support: EY13022

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1183 - D1115
α-Crystallin and Cytochrome C Form a Mitochondrial Complex With MsrA in Lens Cells
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Biomedical Sciences, Florida Atlantic University, Boca Raton, FL.

Purpose: Oxidation of α-crystallin and cytochrome c (cyt c) at specific methionine residues results in loss of their respective chaperone and oxidoreductase activities. Both α-crystallin and cyt c are oxidized in the lenses of Methionine Sulfoxide Reductase A (MsrA) knockout mice in vivo and their activities are dependent on repair by MsrA. Both cyt c, α-crystallin and MsrA are localized to lens cells suggesting they may form a functional complex in specific lens cell sub-types. Here, we examined possible interactions between α-crystallin and cyt c in lens cells as a first step towards establishing a possible role for α-crystallin and MsrA in modulating the apoptotic and oxidoreductase activities of cyt c.

Methods: Levels of cyt c and α-crystallin (total, α A and β B-crystallin) were examined by RTPCR and western analysis of microdissected mouse and human lenses. Protein complexes were analyzed by co-immune precipitation analysis of extracts from cultured human lens epithelial cells (HLEB3 and SRA04/01) and whole human lenses. Protein complexes were further examined by microdissection of whole human and mouse lenses followed by immunofluorescent microscopy and organelle imaging.

Results: As expected, high levels of α-crystallin, MsrA and cyt c were detected in the lens epithelium. Unexpectedly, significant levels of cyt c were also detected in lens fibers. MsrA, cyt c and α-crystallin formed stable complexes that were detected in all lens tissues. These complexes preferentially resided in lens mitochondria.

Conclusions: These results suggest that MsrA, α-crystallin and cyt c form complexes in lens epithelia and fibers. They suggest that both the oxidative stress repair activity of MsrA and the chaperone function of α-crystallin may modulate the activity of cyt c in the lens affecting both lens mitochondrial function, apoptosis and possibly lens differentiation.

CR: R. McGeal, None; W. Lee, None; M. Demos, None; L. Brennan, None; M. Kantorow, None.
Support: NIH grant EY13022

1185 - D1116
Sigma-1 Receptor Stimulation Provides Protection Against Oxidative Damage Through Suppression of ER Stress Responses in the Human Lens
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School of Biological Sciences, University of East Anglia, Norwich, United Kingdom; Biological Sciences, Oakland University, Rochester, MI.

Purpose: Sigma-1 receptor binding affinity has been reported to be increased under oxidative stress. Sigma receptors are mainly expressed in the Endoplasmic Reticulum (ER) membrane and are reported to have regulatory actions on calcium signalling. In the present study we used cells and tissue from the human lens to elucidate the relationship between sigma-1 receptor, ER stress and oxidative stress induced damage.

Methods: The current study employed whole human lens cultures isolated from donor eyes in conjunction with the human lens cell line FH1L24. Cell viability was determined using the MTS assay. Western blot methods were used to assess expression of Sigma-1 receptor and ER stress proteins (Bip, IRE1, ATF6 and p82E2). Apoptosis was detected using western blotting techniques to determine changes in protein expression of pro-Caspase-3 and 12; the TUNEL assay; through detection of LDH leakage into the bathing medium.

Results: Exposure of the human lens cell line FH1.24 to increasing doses of H2O2 led to reduced cell viability. Moreover, reduction in pro-forms of caspase 12 and 3 were observed in response to H2O2. In response to 30 µM H2O2, level of Bip, ATF6, IRE1 and p82E2 were significantly increased within 4 hours of exposure. Moreover, sigma-1 receptor expression was markedly increased in response to H2O2-induced oxidative stress. Application of 30 and 30 µM (+)-pentazocine, a sigma 1 receptor agonist, significantly inhibited the H2O2 induced cell death. In addition, the oxidative stress induced reduction of pro-caspase 3 and 12 was suppressed by (+)-pentazocine. Moreover, the induction of ER stress proteins Bip and IRE1 following oxidative insult were also suppressed by (+)-pentazocine. When applied to cultured human lenses, (+)-pentazocine again demonstrated protection against H2O2-induced opacity, LDH release and apoptotic cell death.

Conclusions: Stimulation of the sigma-1 receptor provides significant protection against oxidative damage. This protection is likely to result from a suppression, but not ablation of the ER stress response. Stimulation of Sigma-1 receptor is therefore a putative therapeutic approach to delay the onset of cataract.

CR: L. Wang, Patent application in process, P; J.R. Reddan, None; J.M. Wormstone, Patent application in process, P.
1187 - D1119
Screening for Ocular Phototoxicity of Fluoroquinolone Antibiotics
Purpose: To determine the potential ocular phototoxicity of fluoroquinolone antibiotics (FQ) used for ocular infections.
Methods: Human lens epithelial cells were exposed to 0.125 - 1 mM FLQs (ciprofloxacin (Cipro), levofloxacin (Lome), nalidixic acid (Nalid), norfloxacin (Norf), and ofloxacin (Oflox)) and 1 µM UV radiation. FLQs and UV were co-incubated with mitochondrial (MitoTracker Orange CM(TMRos) and lysosomal (LysoTracker Red DND-99) probes. Cell viability was measured by MTS and membrane damage as determined by the LDH assay. Flow cytometry was used to determine the extent of necrosis and apoptosis. Binding of the FLQs was determined by shifts in the UV-VIS absorption spectrum of the drugs in the presence of alpha crystallin.
Results: All FLQs tested absorbed light in the UV-A region (320-340 nm). Neither FLQ-drug exposure alone nor UVA exposure alone reduced cell viability. In contrast, cells exposed to UVA and FLQs underwent necrosis and apoptosis and loss of cell viability as measured by MTS and membrane damage as determined by the LDH assay in the order: Cipro > Nor > Lome > Ofx > Nalid. None of the drugs tested were found to bind to alpha crystallin.
Conclusions: FLQs are potentially phototoxic to the eye. However since these antibiotics do not bind to lens proteins, permanent damage to the eye can be prevented if patients protect their eyes from intense sunlight by wearing wrap-around UV filtering sunglasses while taking these medications.
CR: J.E. Roberts, None; C.F. Chigell, None; U.P. Andley, None; B. Zhao, None.
Support: Intramural Research Program of the NIH, National Institute of Environmental Health Sciences

1188 - D1120
Cataractous Lens for Space Radiation Biodosimetry in Mice
Purpose: To elucidate thermal transport in the anterior chamber by visualizing aqueous humor convection.
Methods: Exp. 1: Depth of penetration by 18 or 40 GHz radio frequency (RF) was calculated by measuring complex permittivity of ocular tissue (cornea, aqueous humor, lens, and vitreous). Exp. 2: Rabbit cornea, lens and vitreous temperatures before or during exposure to 18 or 40 GHz RF were measured by thermocouple. Exp. 3: Microencapsulated Thermochromic Liquid Crystal (MTLC) which changes color according to temperature was injected into the anterior chamber prior to exposure then images were recorded by video camera during 18 or 40 GHz RF exposure.
Results: Exp. 1: Estimated ocular penetration depths of 18 and 40 GHz were 1.25 mm and 0.59 mm respectively. Exp. 2: The cornea and lens temperatures before exposure were 32.7±1.3°C and 37.4±1.0°C respectively (p<0.5). Temperature rises were measured to 11°C (Cornea), 8°C (Crystalline lens), and 13°C (Vitreous) were caused by 3 min exposure to 40 GHz of 200 mW/cm². About twice incident power density was necessary under 18 GHz exposures to obtain similar temperature rises. Exp.3: Temperature rise in 18 GHz exposed eyes was first observed in the upper portion of the eye followed by the lower. Both upper and lower high temperature zones gradually moved into the central pupillary area. The thermal transport route in 40 GHz exposed eyes was observed to rise from the right under the cornea and the convection descended to the iris or the lens side.
Conclusions: There is a possibility that the influence on the lens is different in each wavelength. Frequency response and convection of aqueous humor are involved in thermal transport in eyes suffering electromagnetic induced heat ocular damage.
CR: M. Kojima, None; Y. Yamashiro, None; T. Sakai, None; Y. Suzuki, None; K. Sasaki, None; H. Sasaki, None.

1189 - D1121
Modeling Space Radiation Cataract Risk Reduction by Antioxidants and Ontario Ginseng Extract
Purpose: Using a model in vitro lens incubation system to investigate the possible reduction of cataract risk for astronauts, jet crews and radiation accident workers by dietary agents and formulations containing antioxidants and Ontario ginseng.
Methods: Pig eyes obtained from a local abattoir were dissected aseptically and the lenses incubated in medium M99 without serum for 4 days to stabilize. Those with protein leakage less than 10 mg/L were taken for further testing. Incubation media containing MTCA (a glutathione precursor, 2RS)-methylhexahalide-4-β-carboxylic acid. 1 µM or two mixtures beta-carotene (1 µM), ascorbate (1 µM), and either pyrroclavine (5 µg/ml), BCP, or a dried alcoholic extract of Ontario ginseng (BCG, 5 mg/ml) were tested. Lenses were exposed to radiation stress by exposure to 74 MeV protons, or a neutron spectrum similar to the atmospheric spectrum of protons or neutrons, while BCG increased the cataract grade at doses of 1 and 2 Gy radiation. Cataracts were scored using the ScantoxTM 24 hr after radiation. Ascorbate decreased the cataract grade determined by ScantoxTM 24 hr after radiation if they were treated with BCP.
Results: BCP decreased the cataract grade determined by ScantoxTM 24 hr after radiation by 20% while BCP treated lenses showed elevated protein leakage into the medium after both 24 hr and 5 days of incubation following proton irradiation and slightly higher leakage after neutron irradiation. Hsp70 after 7 days incubation were 1.5 fold higher compared with non-irradiated controls. Hsp27 after 24 hr.
Conclusions: Additions of antioxidants may lead to increased risk at low doses (1-2 Gy) of proton irradiation. MTCA-treated lenses showed elevated protein leakage into the medium after both 24 hr and 5 days of incubation following proton irradiation and slightly higher leakage after neutron irradiation. Hsp70 after 7 days incubation were 1.5 fold higher compared with non-irradiated controls. Hsp27 after 24 hr.
CR: J.R. Trevithick, None; T.D. Dzialoszynski, None; E.G. Noble, None.
Support: Canadian Space Agency, Ontario Ginseng Research and Innovation Consortium

1190 - D1122
Non-Invasive Quasi- Elastic Light Scattering (QLS) Assessment of the Pre- Cataractous Lens for Space Radiation Biodosimetry in Mice
Purpose: The goal of this project is to investigate the natural history of Rayleigh light scattering changes in pre- cataractous lenses of mice exposed to high-energy proton or iron particle radiations prevalent in space. A secondary goal was to assess the efficacy of a purpose-designed quasi-elastic light scattering (QLS) instrument as a non-invasive ocular biodosimetry platform for detecting and monitoring exposure to biologically relevant space radiation.
Methods: C57B6 male mice (25 males/group) were irradiated with 10 or 100 cGy of 130 GeV/amu protons (LET=0.22 keV/µm) or 1 GeV/amu iron ions (LET=155 keV/µm) in the NASA Space Radiation Biomechanics and Neurology (SRNB) Control mice (n=10 males) were sham irradiated. For each examination, the left eye was dilated (1% tropicamide) and the lens assessed by QLS without anesthesia. Each examination consisted of 15 autocorrelation acquisitions with infrared slit-lamp imaging of the sampled lens region. Autocorrelation and scattering intensity analyses were conducted on data averaged over each 15 acquisition test session and compared to conventional slit lamp biomicroscopy conducted on the same mice. Baseline QLS was conducted on all mice prior to irradiation. Polystyrene bead (0.12, 0.1, 0.05 µm) were used for QLS instrument calibration.
Results: We demonstrated intra-assay precision of the QLS platform and successfully acquired longitudinal autocorrelation function and Raleigh scattering intensity values from non-anesthetized mice in irradiated and sham cohorts. QLS and slit-lamp examinations were compared.
Conclusions: Non-invasive QLS instrument for in vivo lens assessment has been successfully deployed to study the longitudinal effects of space radiation exposure in mice. This work is supported by the NASA Office of Life and Microgravity Science and Applications (NASA OLSA) and the National Institute on Aging (NIA) Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.
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Support: NASA
1212 - 8:30AM

Lens Cell Proliferation, Differentiation and Development Require K6 on Ubiquitin

Laboratory for Nutrition and Vision Research, Tufts University; Human Nutrition Research Center on Aging, Boston, MA; Orth’p & Vis Sci & Genetics, Albert Einstein Coll of Medicine, Bronx, NY; Lab of Molecular & Dev Bio, National Eye Inst/NIH, Rockville, MD; Ophthalmology, Rush University Medical Center, Chicago, IL; Department of Chemistry and Biochemistry, University of Maryland, College Park, MD.

Purpose: Many cellular processes, such as cell cycle control, differentiation, signal transduction, transcription and removal of obsolete proteins are regulated by ubiquitin, a proteasome pathway (UPP). Surprisingly little is known about the ubiquitin molecule itself. There are 7 lysines on ubiquitin (Ub). The least understood is K6. For degradation of a substrate to proceed, multiple Ub, often linked to each other in isopeptide bonds which utilize K48 of one ubiquitin and the carboxyl terminus of another, are attached to the substrate.

Methods: In previous studies, in order to investigate the role of Ub in lens proliferation we expressed K6W-UB (a mutant Ub that precludes lysine 6 linkages on ubiquitin and acts as dominant-negative inhibitor of the UPP) in human lens epithelial cells under control of an α-crystallin promoter.

Results: We found that cell proliferation is inhibited, mainly due to the delay of the cell cycle at the G1/M phase and impaired UPP-dependent degradation. Mice which express higher levels of K6W-UB, even against endogenous wt ubiquitin, show severe cataracts with accumulation of high mass K6W-UB containing conjugates, protein aggregation and decreased protein degradation. Newer data indicate that K6W-UB expression inhibits lens cell proliferation and lens fiber differentiation, specifically delayed gamma-crystallin expression. This delay is paralleled by phosphorylation of lamins, disassembly of the nuclear membrane and entry of DNase II into the nucleus. Thus, fibers are abnormal and demucleation is impaired.

Conclusions: This data establishes an unknown role for Ub specifically on K6 on Ub, in lens differentiation (deenucleation), development and homeostasis.

CR: A. Caceres, None; F. Wang, None; E. Dudek, None; O. Avidan, None; A. Cvekl, None; Y. Yang, None; E.F. Wawruszewski, None; J.R. Kuszak, None; D. Fushman, None; A. Taylor, None.

Support: NIH EY ROI 13250, Am Health Assistance Foundation, Johnson + Johnson

1214 - 9:00AM

α6 Integrin Transactivates an IGF-1R-Mediated Survival-Signaling Pathway

Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA.

Purpose: Our previous studies show that the canonical mitochondrial death pathway has a crucial role in signaling lens differentiation initiation through the activation of the caspase-3 protease. The ability of this pathway to function as a molecular switch in lens differentiation depends on the concurrent induction of the survival molecules that regulate it, including proteins in the Bcl-2 and the Inhibitor of Apoptosis Protein (IAP) families. Here, we studied the upstream signaling pathway that induces expression of these survival molecules, focusing our investigation on α6 integrin as the transactivator of an IGF-1R-mediated survival-signaling pathway involving Nuclear Factor Kappa B (NFκB) activity.

Methods: α6 integrin receptor was knocked down in primary quail lens cells with a siRNA approach and activated by exposure to PMA. Expression of α6 integrin, activation of IGF-1R, and expression of its downstream effectors including Nfkb, Bcl-2, and IAP1/2 were determined by Western blot analysis. Receptor complexes in differentiation-specific fractions of the E10 chick lenses were analyzed by co-immunoprecipitation.

Results: As lens cells initiate their differentiation program in the equatorial zone IGF-1R is recruited to and activated in α6 integrin receptor signaling complexes. The suppression of IGF-1R activation following siRNA knockdown of α6 integrin showed that activation of IGF-1R was dependent on α6 integrin. In support of this finding the activation of α6 integrin by PMA induced activation of IGF-1R. The expression of Bcl-2 and IAP survival proteins in lens cells is dependent on an IGF-1R/IR signaling pathway. This survival pathway was found to be dependent on expression of α6 integrin. Knockdown of α6 integrin suppressed expression of Nfkb/Rela and its effectors Bcl-2, -IAP1/2 and survivin, and these lens cells failed to differentiate, as evidenced by inhibition of the expression of filensin, MIP2 and p27.

Conclusions: α6 integrin transactivates an IGF-1R-mediated survival-signaling pathway that is crucial for the initiation of lens cell differentiation.

CR: S. Basu, None; J.L. Walker, None; L. Zhang, None; A.S. Menko, None.

Support: EY 014796/EY010577

1215 - 9:15AM

Expression of Fiber-Specific Proteins is Regulated by Post-Transcriptional Mechanisms: A New Paradigm for Lens Fiber Cell Differentiation

Ophthalmology & Visual Sci, Washington Univ, St Louis, MO; “Biologic & Materials Sciences, Univ Michigan, Ann Arbor, MI; Dept of Human Molecular Genetics, Tel Aviv University, Tel Aviv, Israel.

Purpose: Lens transparency depends on the production of large amounts of “fiber-specific” proteins. The control of gene and protein expression in fiber cell differentiation is incompletely understood.

Methods: Laser microdissection was used to isolate E9.5 lens placode cells from wild type (WT), P.parvus (Parv)−/−, Le-Cre conditional knockout (CKO) and BMP receptor CKO (Bmpr1a/c/cre/cre)embryos and cortical fiber cells from one-month-old mice. Epithelia from 1- to 8-month-old WT lenses were separated into central and peripheral regions. RNA was amplified (NuGEN Ovation kit) and used to probe Illumina whole genome microarrays. Transcripts were detected and quantified by PCR and localized by in situ hybridization. Proteins were detected by western blotting and confocal microscopy.

Results: αβ is the only crystallin previously identified in the mammalian lens placode. Microarray analysis showed that WT placode cells accumulate transcripts encoding a subset of αβ crystallins (Crystb, Crystc, and d) and the “fiber-specific” protein, MIP. These RNAs were greatly reduced in Parv or BMP receptor CKO plakodes. Adult lens central epithelial cells had abundant transcripts encoding most “fiber-specific” proteins, including Crystb1, 2 and 4, Crystb1-C, Crystd, e, f, and i transcripts were less abundant in central than in peripheral epithelial cells. Crystg, c, and f transcripts decreased with age in the epithelium, but increased markedly in fiber cells. Other “fiber-specific” mRNAs were present at similar levels in epithelial and fiber cells. MIP transcripts were among the most abundant in epithelial cells, although MIP protein was not detected in these cells. Similarly, Prox1 mRNA was abundant in central epithelial cells, but Prox1 protein was detected only in transition zone epithelial cells and in fiber cells. Prox1 staining was prominent in the nuclei of all epithelial cells from lenses lacking the BMP receptor, Acvr1.

Conclusions: It is generally thought that fiber-specific genes are expressed at the onset of fiber cell differentiation. Instead, some “fiber-specific” genes are actively expressed before the control of Parv and BMP signaling in the placode. Most “fiber-specific” transcripts are already abundant in adult epithelial cells. Synthesis of MIP and Prox1 proteins is controlled by differential translation, a previously unrecognized mechanism regulating fiber cell differentiation.

CR: D.C. Beebe, None; J. Huang, None; L.A. Wiley, None; A. DeMaria, None; Y. Liu, None; Y.-B. Shui, None; L.K. Dattilo, None; V.M. Kaartinen, None; R. Ashery-Padan, None; S. Basu, None.

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1217 - 9:45AM
Racl Conditional Knockout Mice Reveal a Crucial Role for Rac GTPase in Regulating Lens Fiber Cell Migration, Polarity, Suture Formation, and Lens Shape


Purpose: To evaluate the involvement of Rac GTPase in lens fiber cell migration, polarity, adhesive interactions and cytoskeletal organization during the lens development.

Methods: Rac1 GTPase lens conditional knockout (cko) mice were generated using Rac1floxflox and Cre transgenic mice expressing the Cre recombinase at different developmental stages and in different regions of the lens. Embryonic, neonatal and postnatal lenses derived from the Rac1 cko mice were characterized histologically, immunohistochemically and biochemically.

Results: The Rac1 cko mice derived from Le-Cre and MLR-10 transgenic mice, which express Cre in both the lens epithelium and fibers starting from embryonic day E8.75 and E10.5, respectively, exhibited defective lens integrity and a marked reduction in eye weight. The lenses of Le-Cre and MLR-10 Rac1 cko mice revealed a predominant phenotype characterized by abnormal fiber cell migration and orientation, with the terminals of basal and apical fibers exhibiting a striking, outwardly projecting arrangement starting at day E15.5 and E17.5, respectively. The Rac1 cko lenses derived from MLR-39 mice expressing Cre only in the lens fibers demonstrated a moderate reduction in eye weight with abnormal fiber cell migration starting from day 1. While Rac1 cko lenses exhibited shape abnormalities and defective suture formation, fiber cell differentiation and epithelial proliferation were unaffected in such lenses. As well, Rac1 cko lenses were characterized by defective actin cytoskeletal organization concomitant with increased G-actin levels and cofilin phosphorylation, decreases in levels of phospho-paxillin, arp2/3, WAVE2 and spectrin staining, and alterations in distribution of cell adhesive molecules including β-catenin, N-cadherin, Abi, Rap1 and Nectin. The levels of integrins including αvβ3, α5β1, and β3 were markedly reduced in the Rac1 cko mouse lenses.

Conclusions: The Rac1 cko mouse lenses demonstrate dramatic abnormalities of organization and migration of fiber cells, and defective suture formation in association with disrupted actin polymerization and cell adhesive interactions. These findings support a vital role for Rac GTPase in regulation of lens fiber cell migration, cytoarchitecture, shape, polarity, and adhesive interactions.

CR: R. Maddala, None; B. Chauhan, None; C. Walker, None; C. Morris, None; Y. Zheng, None; M.L. Robinson, None; R.A. Lang, None; F.V. Rao, None.
Support: EY12201, EY018590 (PVR) and EY017848 (RAL)

1218 - 10:00AM
The Cdk5 Activating Protein P39 Directly Links Muskelin to Myosin and Stress Fibers

B.K. Tripathi, P.S. Zelenka. LMDLB, National Eye Institute, Rockville, MD.

Purpose: We have recently shown that opposite ends of p39, a cyclin dependent kinase (CDK) activating protein, bind myosin essential light chain (MLC) and the LisH domain protein, muskelin, both of which are known to affect cytoskeletal organization. Here we seek to determine whether p39 specifically links muskelin to myosin and the significance of these interactions to cytoskeletal organization in lens.

Methods: Human lens epithelial cells were allowed to spread on fibronectin for 2 hr. Immunoprecipitation and immunoblotting with specific antibodies were used to confirm the interaction of endogenous proteins in lens epithelial cell lines and whole rat lens extracts. Cdk5 activity was inhibited by a pharmacological inhibitor (olomoucine, 15 µM) and by dominant negative GFP-Cdk5(D144N). Cdk5 and p39 expression were suppressed by specific siRNAs. Lens epithelial cells were transfected with fluorescence-tagged fusion constructs, and subcellular localization of proteins was determined by confocal fluorescence microscopy. RT-PCR primers were designed to detect alternatively spliced isoforms of myosin heavy chain (MHC), MHC II-B and MHC II-B1 (10 amino acid insert).

Results: The alternatively spliced isoform, MHC II-B1, a known substrate of Cdk5, was expressed in lens epithelia and fibers. Immunoprecipitation and immunoblotting of lens epithelial cell lines and rat lens extracts confirmed that endogenous MHC II-B and myosin regulatory light chain (MLRC) formed a complex containing MLC, p39, and muskelin. Suppression of p39 by siRNA significantly reduced co-immunoprecipitation of muskelin with MLC, MLRC, and MHC II-B. Fluorescence-tagged p39 and MLC fusion proteins colocalized along stress fibers and cortical actin filaments. Immunofluorescence microscopy demonstrated that muskelin colocalized with microtubules, MLRC, and contracting stress fibers during cell migration.

Conclusions: p39 is essential for formation of a protein complex connecting muskelin to myosin. The interaction thus targets Cdk5/p39 kinase activity to cytoskeletal substrates such as MHC II-B1 during cell adhesion and migration, and may serve to link microtubules to stress fibers.

CR: B.K. Tripathi, None; P.S. Zelenka, None.
Support: NIH Grant Z01-EY000238-20
3159 - 1:45PM Glutaredoxin 2 (Grx2) Knockout Increases Cellular Sensitivity to H2O2-Induced Cell Injury in Mouse Lens Epithelial Cells
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**Purpose:** Glutaredoxin 2 (Grx2) is an isozyme of thioredoxintransferase (TTase or Grx1) present in the mitochondria but its function is not well understood. The purpose of this study was to evaluate the roles of Grx2 in anti-oxidative and mitochondrial complex I protective functions in the lens by using Grx2 knockout mouse lens epithelial cells (LECs) as a model.

**Methods:** Primary cultures of LECs were established from the lenses of wild-type (WT) and Grx2-knockout (Grx2−/−) mice. Cells were probed for α-A-crystallin, Grx2 by Western blot analysis while cell viability was examined by WST-8 assay. Glutathione (GSH) level, Lactate dehydrogenase (LDH) release, Grx2 activity and Complex I activity were determined by spectrophotometric assays. Reactive oxygen species (ROS) was detected using DCF-DA fluoroscein with a cell sorter. Apoptosis was quantified by flow cytometry.

**Results:** LECs identiﬁed was confirmed by positive immunoreactivity to anti-αA-crystallin antibody. Western blotting showed normal expression of Grx2 in WT cells but not in Grx2−/− KO cells. KO cells had only trace Grx2 activity (15%) in comparison with the WT, while cells showed similar morphology and growth pattern, and contained same level of GSH and complex 1 activity in mitochondria. However, KO cells were more sensitive to oxidative stress (100 μM H2O2, for 6 h) and exhibited lower cell viability and LDH leakage compared with the WT cells. In addition, knockdown of Grx2 weakened the cell’s ability to detoxify H2O2 and deteriorated the H2O2-induced complex I activity loss.

**Conclusions:** Grx2 play a major role in protecting mouse LHE cells against H2O2-induced cell injury. The mechanism of this protection is likely associated with its ability to protect cells from electron transport chain and its peroxidase activities.

**CR:** H. Wu, None; K. Xing, None; L.-R. Li, None; E. Giblin, None; Y.-S. He, None; M.F. Lou, None.

**Support:** NIH Grant 1R01EY10595

3159 - 2:15PM Roles of Multiple Genetic Factors in Nuclear Cataracts
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**Purpose:** To study the molecular basis of nuclear cataracts linked to the mutations of connexin and gamma-crystallin genes and to identify how other genes regulate the severity of nuclear cataracts. Elevated alpha3 connexin enhances lens cell-cell communication that likely prevents calcium level elevation to inhibit the calpain-mediated degradation of crystallin. Mutation of alpha3 suppressor in alpha3 knockout mice, we located this dominant suppressor in a region close proximity to the marker D7Mit115. The light scattering measurement of various pathological processes including crystallin degradation and fiber cell degeneration. In addition, the severity of nuclear cataracts was variable among alpha3 knockout mice and is induced in lens cells upon oxidative stress treatment. Oxidation of methionines in both alpha-crystallin and cytochrome c leads to loss of function. TXNL6 was found in both epithelial and fiber cells of the human lens and is induced in lens cells upon oxidative stress treatment. Oxidation of methionines in both alpha-crystallin and cytochrome c leads to loss of function. TXNL6 acted as a reducing system for MsrA repair of oxidized methionines of alpha-crystallin and cytochrome c resulting in restoration of their respective chaperone and oxidoreductase functions.

**Conclusions:** Oxidation of methionines in key proteins during aging and oxidative stress likely plays a significant role in lens aging and cataractogenesis. The novel approaches to identify the system identified here; TXNL6, likely serves to activate the lens repair activity of MsrA and possibly other important lens repair systems requiring thioredoxin-like proteins. Increased TXNL6 expression upon oxidative stress and aging likely serves to aid MsrA in the repair of oxidized alpha-crystallin, cytochrome c and other key lens proteins to avert cataract formation.

**CR:** L.A. Brennan, None; W. Lee, None; R. McGreal, None; L. David, None; M. Kantorow, None.

**Support:** NIH Grant 1R01EY13022

3159 - 2:45PM Histamine Stimulates Multiple Signalling Pathways in Human Lens Cells Leading to Accelerated Growth Rate
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**Purpose:** Histamine is an important inflammatory molecule. Following injury to the eye, such as cataract surgery, levels are likely to rise and could contribute to the development of posterior capsular opacification. Consequently, a greater understanding of the effect of histamine on signalling pathways and growth rates of human lens cells.

**Methods:** Histamine receptor gene expression in the native human lens and the human lens cell line HFL 124 was determined by gene array analysis. Calcium signalling was investigated through imaging of Flura-2 loaded cells while phosphorylation of ERK, p38 and JNK in response to histamine was assessed using the BIOplex suspended bead array system. Growth rate was assessed in HFL 124 cells by changes in protein level detected by coomassie blue dye extraction and 2H-thymidine incorporation. In addition, the human capsular bag model was deployed in this system, cell growth was measured as coverage of the central posterior capsule.

**Results:** Analysis of array data from the human lens revealed only the H1-receptor sub type is detectable; this is exclusively expressed in the epithelial cell population. Moreover, this pattern of expression is conserved in the HFL 124 cell line. Application of histamine to HFL 124 cells produced a dose-dependent increase in intracellular calcium, with detectable responses observed from 1μM histamine. Addition of 100μM histamine induced significant elevation of phosphorylated ERK and p38; pJNK was also elevated, but this change was not significant. In each case, peak response was observed 10 minutes following stimulation. Addition of histamine (30μM) significantly stimulated HFL 124 cell growth. Moreover the rate of cell coverage of the posterior capsule in capsular bags was accelerated by 10μM histamine; this response was inhibited by 10μM triprolidine (H1-receptor antagonist).

**Conclusions:** The dominant histamine receptor present in lens epithelial cells is the H1-receptor sub-type. Activation of this receptor induces significant changes in calcium and MAPK signalling pathways. In addition, histamine stimulates an accelerated cell proliferation that could contribute to the incidence of PCO proliferation; therefore, histamine receptor antagonists could provide therapeutic benefit following cataract surgery.

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See page 1590 for abstract number 1590- 1597.
Monday, May 3, 1:45 PM - 3:30 PM  Room 315  Paper Session  Program Number Range: 1594 - 1599
249. Protection and Repair Mechanisms  Organizing Section: LE

Aggregation of C-Terminal Truncated Human AlphaA-Crystallins in Mammalian Cells and Protection by AlphaB-Crystallin


Purpose: Post-translational modifications in human eye lens crystallins such as the formation of C-terminal truncated alphaA-crystallins, alphaA-Cr, alphaA-II and alphaA-III is believed to play a role in the development of senile cataract. The current investigation was aimed to study the fate of the truncated alphaA-crystallins and their interactions with native alphaA- and alphaB-crystallins in situ in living mammalian cells using fluorescence resonance energy transfer (FRET) and laser scanning confocal microscopy (LSM).

Methods: Human alphaA-wt and alphaB-wt genes were cloned into pAmCyan1-Cl (CFP) vector for expression in cyan color. Genes of alphaA-Δ70 and the C-terminal truncated alphaA crystallins, alphaA-Δ70, alphaA-Δ, and alphaA were cloned into pZsYellow1-C1 (YFP) for expression in yellow color. YFP tagged alphaA-wt or each of the truncated alphaA-crystalin was individually transfected or co-transfected with CFP tagged alphaA-wt or alphaB-wt into HeLa cells and after 48h, cells were examined by laser scanning confocal microscopy. Proportion of cells having significant level of protein aggregates was determined by visual assessment and confirmed by subsequent light scattering studies. To show whether C-terminal truncation of alphaA-crystallin affects protein-protein interaction with native alphaA- or alphaB-crystallin, FRET-acceptor photo-bleaching protocol was followed.

Results: When YFP tagged alphaA-wt, alphaA-Δ70, alphaA-Δ, and alphaA were expressed individually, nearly 5, 35, 70, and 85% cells, respectively, showed significant level of protein aggregates and also associated with distorted cell morphology only in cells harboring truncated alphaA-crystallins. Co-expression with alphaA-wt failed to inhibit protein aggregation in cells expressing alphaA-Δ70 or alphaA-Δ, Cells expressing alphaA-Δ70 appeared normal and lacked protein aggregates. Co-expression with alphaB-wt significantly improved cell morphology and decreased protein aggregation. FRET studies showed that the interaction of alphaA-wt with alphaA-wt or alphaB-wt was much stronger than that of alphaA-Δ70 and alphaA-Δ. Moreover, the overall interaction of C-terminal truncated alphaA-crystallins with alphaB-wt was stronger than the interaction with alphaA-wt.

Conclusions: This is the first report showing that C-terminal truncated alphaA-crystallins tend to aggregate in living mammalian cells unless they maintain strong interaction with native alphaB-crystallin. Association with alphaB-crystallin provides protection to the truncated alphaA-crystallins.

Support: NIH Grant EY11352

Mini-αA-Crystallin Prevents Oxidation of Ascorbic Acid by Cu²⁺

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Purpose: Previous studies have shown that small heat shock protein α-crystallin binds Cu²⁺. The Cu²⁺ interaction with α-crystallin affects the chaperone activity. Earlier, we reported that αA70-88 peptide (also known as αA-mini-chaperone) can independently function as chaperone molecule. The present study was undertaken to determine whether mini-αA-crystallin possesses Cu²⁺ binding property and inhibits copper induced oxidation of ascorbic acid.

Methods: Mini-αA [DFVIFLDVKHFSPEDLTVK] was supplied by GenScript Corporation. Cu²⁺ solution was prepared in buffer with glycin to avoid non-specific interaction. The recombinant αA-crystallin was expressed in E. coli cells and purified using chromatographic techniques. Deletion mutant αA-Δ70-77 was created by site directed mutagenesis method. Cu²⁺-mediated oxidation of ascorbic acid was measured as change in absorbance at 260 nm in a spectrophotometer. Secondary structure of mini-αA was analyzed by CD spectroscopy in presence or absence of Cu²⁺. Bis-ANS interaction with peptide was followed by fluorometry.

Results: Four hundred μM of ascorbic acid was completely oxidized by 1μM of Cu²⁺ in 50mM phosphate buffer pH7.2 in 20 min. Addition of 33 nanomole of mini-αA-crystallin completely suppressed Cu²⁺-induced oxidation of ascorbic acid. The recombinant protein αA-Δ70-77 showed 80-90 percent reduced protection against Cu²⁺-mediated oxidation of ascorbic acid, whereas wild-type αA-crystallin completely prevented the oxidation of ascorbic acid during the experimental period. The binding of Cu²⁺ to mini-αA-crystallin increased the ellipticity of the peptide in far-UV region during CD spectroscopy. Prior binding of Cu²⁺ to the peptide resulted in diminished bis-ANS interaction with mini-αA-crystallin.

Conclusion: The present study demonstrates that the 70-77 region in αA-crystallin is a Cu²⁺ binding site. Mini-αA-crystallin prevents Cu²⁺-induced oxidation of ascorbic acid.

CR: M. Raja. None; P. Santhoshkumar. None; K. Sharma. None.
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2347 - D786
Anoikis Regulated by PKD and Bit1 May Be Involved in Lens Denucleation
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Purpose: In the Nuc1 spontaneous mutant rat, an unusual feature is the failure of the normal programmed loss of nuclei from lens fiber cells. We have utilized the mutant to investigate the possible role of PKD and Bit1, as anoikis effectors, in normal lens denucleation process.
Methods: Immunofluorescence staining was performed on frozen sections of both wildtype (wt) and Nuc1 rat lenses. Various stages examined include embryonic (E) day 19, postnatal (P) day 3 and 10.
Results: At E19, both wt and Nuc1 lenses show a similar expression pattern for PKD. The cells in the bow region of the lens and fiber cells at the center of the lens were positive with no expression of PKD detected in the lens epithelium. At P3, wt lenses lost PKD expression in the central fiber cells with weak expression remaining at the equator. A similar pattern was observed at P10, with further decrease in staining at the equator. In contrast, in Nuc1 homozygous lenses the staining at P3 and P10 was similar to that observed in E19 Nuc1 lenses. Bit1 was expressed both in fiber cells and epithelium at E19. In wt, Bit1 decreased in fiber cells after birth similar to PKD. In contrast, there was no apparent decrease in fiber expression in the Nuc1 lenses at P3-P10.
Conclusion: Programmed removal of nuclei from the lens fiber cells is thought to occur by a process similar to apoptosis. Anoikis mediated by PKD and Bit1 is apoptosis induced by loss of cell attachment to the extracellular matrix. Our present studies indicate that PKD and Bit1 expression is lost from wt lens fibers during development along with the loss of cell nuclei. In Nuc1 homozygous lens fibers, where nuclei are retained, PKD and Bit1 continue to be expressed after birth. We provide novel evidence that anoikis regulated by PKD and Bit1 may be involved in lens denucleation.
CR: B. Ma, None; C. Zhang, None; D. Sinha, None; S. Zigler, Jr., None.
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2348 - D787
The Formation of a Second Lens in the Mutant Zebrafish, Occhiolino
M. Aose1, T.M.S. Greiling1, H.R. Djadi2, T.H. Linbo3, D.W. Raffle4, J.I. Clark5,6,7,8.
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Purpose: Analysis of abnormal lens development in Occhiolino mutant zebrafish.
Methods: Zebrafish were treated with N-ethyl-N-nitrosourea (ENU) and offspring were screened for eye phenotypes. Zebrafish were euthanized in 0.4 mM tricaine; fixed in 4% paraformaldehyde in 5% sucrose in phosphate buffered saline (PBS); the fixed fish were dehydrated in 15% agarose/5% sucrose/PBS and cryoprotected in 30% sucrose/PBS; then the agarose blocks were mounted in OCT and frozen in isopentane cooled in liquid nitrogen. The fish were cryosectioned for histology and immunofluorescence. H&E staining, BrdU labeling (proliferation) TUNEL labeling (apoptosis) and immunohistochemistry were performed using selected antibodies. For 2-photon imaging, the Occhiolino zebrafish were crossed with the Q01 transgenic fish which express CFP fused to Gap43, a membrane-targeting sequence driven by an EF1α promoter and a hexamer of the DsRed6 enhancer.
Results: The Occhiolino mutant was identified in an F3 screen for mutations affecting zebrafish nervous system development. The eye of the Occhiolino mutant developed normally until 3 days post fertilization (dpf), when visual function was established. After 3 dpf, the epithelial cells in the Occhiolino lens lost spherical symmetry and the ability to proliferate and migrate. The abnormal epithelium became multilayered and a new lens mass formed, sometimes appearing as a second lens between the first lens and the cornea. BrdU and TUNEL labeling demonstrated the cell-cycle was abnormal in the cells of the second lens cell mass. The first lens ruptured and was displaced posteriorly, pushing into the developing retina, occasionally contacting the extra cellular matrix (ECM) of Bruch’s membrane. The retina appeared to expand and decrease the pupil diameter. The first lens was smaller, and the proteins different than in Western populations. Compaction appears to continue throughout life.
Conclusions: The phenotype of Occhiolino mutant zebrafish resulted from abnormal proliferation and migration of the lens epithelium.
CR: M. Aose, None; T.M.S. Greiling, None; H.R. Djadi, None; T.H. Linbo, None; D.W. Raffle, None; J.I. Clark, None.
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2349 - D788
Conditional Deletion of Msx2 in the Developing Lens Leads to Microphthalmia in Mice
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Purpose: Msx form one of the most highly conserved families of homeobox-containing genes. Homeobox genes function as essential transcriptional regulators in a variety of developmental processes. Overexpression of the Msx2 gene in transgenic mice can alter Bmp4 and Bmp7 gene expression, initiate cellular apoptosis in the developing neural retina, and cause microphthalmia. In current experiments, we investigated the function of Msx2 in conditional knockout mice.

Methods: To learn more about the function of Msx2 during lens development, we examined mice in which the Msx2 was selectively deleted in the lens using Cre-loxP-mediated gene targeting.

Results: Msx2-deficient mice show a persistent lens stalk, a delay in lens formation and the microphthalmia at developmental stages. The delay in lens formation is associated with an increased apoptosis activity in the lens placode during its invagination into the optic cup. For lens lineage-specific transcriptional factors, the expression of FoxE3 is reduced but no obvious changes in Fast, Sox2 and Atp2 expression are observed in the developing lens of Msx2-deficient mice compared to the wild-type littermates. The expression of FoxE3 in early eye development is under control of Msx2, and reduced expression involved in adhesion between lens and cornea.

Conclusions: Our research results showed the Msx2 is critically required during the morphogenetic processes of early eye development.

CR: Z. Yu, None; J. Zhao, None; J. Zhang, None; Y.-H. Liu, None; S. Yiu, None; Y. Lallemand, None; V. Bensoussan, None; L. Zha, None.

Support: EY015417

2351 - D790
Lens Fiber Cell Morphology is Affected by Sip1 Conditional Deletion
A.L. Grabitz, D.A. Scheiblin, M.K. Duncan. Department of Biological Sciences, University of Delaware, Newark, DE.

Purpose: Sip1, or Smad interacting protein 1, is a member of the Zeb protein family and is a known binding partner of Smad proteins that co-regulate TGFB induced epithelial-mesenchymal transition (EMT). Sip1 is critical for the separation of the lens vesicle from the head ectoderm and the later expression of γ-crystallin in lens fibers. This work tests the hypothesis that Sip1 plays a role in lens fiber cell differentiation and morphogenetic processes of early eye development.

Methods: Mice homozygous for the Sip1-/- allele were bred with mice carrying the MLR10 Cre gene, which is expressed throughout the lens vesicle. Gene deletion was assessed by immunofluorescent localization of Sip1 and FCRE. The structure of lenses lacking Sip1 was assessed by scanning electron microscopy (SEM) and conventional histological methods. Crystallin expression was investigated using gel electrophoresis and protein staining. C57B6 mice were used as the wild type controls.

Results: Conditional deletion of Sip1 results in profound defects in the shape and structure of lens fiber cells beginning at approximately the lens vesicle stage of embryonic development. Additionally, conditional deletion of Sip1 results in fiber cells with a very disordered ball and socket structure in comparison to controls. Crystallin expression, however, is not dramatically altered upon the loss of Sip1.

Conclusions: These data indicate that Sip1 is important for lens fiber cell differentiation and fiber cell structure. However, our data suggest that Sip1 is not essential for crystallin expression in the lens. Future work will investigate the targets of Sip1 in the lens and how these targets regulate lens structure.

CR: A.L. Grabitz, None; D.A. Scheiblin, None; M.K. Duncan, None.

Support: EY12221

2352 - D791
Uhrf1 and Dnmt1 Function is Required for Vertebrate Lens Development
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Purpose: DNA methylation results in epigenetic transcriptional repression, and is required for the lens vesicle to detach from the surface ectoderm during early anterior segment morphogenesis. During this process, the expression of several genes, including α-crystallin in lens fibers.

Methods: DNA methylation in terminal differentiation and organogenesis has so far been studied in very few cell types, due in part to the early embryonic lethality of knockout mice lacking genes required for DNA methylation. Unlike mouse models, zebrafish with mutations in two key epigenetic regulators, DNA Methyltransferase 1 (dnmt1) and Ubiquitin-like, Containing PHD and RING Finger Domains 1 (uhrf1), survive to late embryonic stages, at which time many complex organs (including the eye) have formed. We have utilized these mutant zebrafish lines to study the role of DNA methylation in lens development.

Methods: Lens formation has been assayed using histological and immunohistochemical techniques. In situ hybridizations have been performed to identify relevant mRNA distribution in the eye. Genomic DNA methylation has been assayed using a Southern blot and by comparison of methylation-sensitive and insensitive restriction enzyme cleavage. To study lens- and cell-autonomy of the phenotype, whole lens transplants and shield-stage transplants (used to generate embryos with mosaic lenses) have been performed.

Conclusions: Loss of Dnmt1 and/or Uhrf1 function leads to morphologically abnormal lenses which contain disorganized and apoptotic lens fibers. Genomic DNA methylation in dnmt1 and uhrf1 mutants is reduced by 75%, however many genes required for lens fiber cell differentiation have reduced expression in mutant lenses compared to siblings, suggesting that decreased DNA methylation does not lead to universal gene upregulation. The results of lens transplant experiments demonstrate that Uhrf1 and Dnmt1 functions are required lens-autonomously, but perhaps not cell-autonomously, during lens development in zebrafish.

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2353 - D792
Negative Regulation of Lens Cell Proliferation and Fiber Differentiation by Sprouty
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Purpose: Numerous growth factors are known to maintain proper lens growth by regulating the proliferation of epithelial cells and their differentiation into precisely organised fibre cells. These growth factors induce such cellular responses by activating various receptor tyrosine kinase signaling pathways that are negatively regulated by different inhibitory molecules, including members of the Sprouty (Spsy) family. In our laboratory, Spsy1 and Spsy2 have been shown to be expressed in the lens, leading to the hypothesis that Spsy may play a role in the regulation of lens epithelial cell proliferation and differentiation. We examined the effect of Spsy on these cellular processes by overexpressing Spsy1 and Spsy2 in lens cells.

Methods: Transgenic mice overexpressing Spsy1 (using the osteo/α-crystallin promoter) or Spsy2 (using LeCre-mediated Spsy gain-of-function) in the lens were generated. The lenses of these mice were characterised using a range of histochemical and immunolabelling techniques. Cells in lens epithelial explants overexpressing Spsy were used to examine the responsiveness of lens cells to growth factor stimulation.

Results: Overexpression of Spsy1 or Spsy2 in the lens of transgenic mice showed similar phenotypes, including a reduced lens size with subsequent posterior lens capsule thinning and rupture. The reduced size of the lens may have resulted from a decreased rate of lens-epithelial cell proliferation and/or reduced rate of secondary fibre differentiation. The ability of cells overexpressing Spsy to differentiate in vitro was also compromised in response to FGF. Interestingly, the ability to influence normal differentiation of lens fibres cells did not appear to be dependent on the continued ability of cells to phosphorylate the MAPKs, ERK1/2.

Conclusions: Constitutive expression of sprouty or sprouty2 in the lens of transgenic mice showed hypoplastic phenotypes in the lens highlights the ability of different Sprays to negatively regulate similar signaling pathways in the lens. The exact signaling pathways influencing lens fibre development are still to be determined. Overall, Spsy may serve to tightly regulate the signalling processes essential for maintenance of lens structure and function.

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Support: NIH Grant EY03177 and NHMR (Australia)

2354 - D793
Excessive Hedgehog Signaling Results in Misexpression of Cell Cycle Proteins and Abnormal Cell Cycle Behaviour During Lens Development
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Purpose: We have previously shown in an activated smoothened (smo) mouse model mimicking excessive Hedgehog (Hh) signaling, that lenses display disrupted patterns of lens cell proliferation and apoptosis. In the current study, we investigated which aspects of the cell cycle are affected in this mutant mouse model.

Methods: An ectodermal cre-recombinase (cre-et) was used to create a conditional activation of smo (gene encoding for the smo receptor in Hh signaling) in the head ectoderm, including the lens placode, of mice to study the effects of constitutive Hh signaling. Eyes and lenses were examined at embryonic day (E) 12.5, 15.5, 16.5 and 18.5 using histological and immunohistochemical techniques.

Results: Previous proliferation and apoptosis studies with PCNA and TUNEL have shown aberrant patterns of cellular proliferation and cell death respectively, beginning after E12.5, indicating possible cell cycle defects in the activated smo mutants. CyclinD1 promotes cell cycle entry, and is normally expressed in the developing anterior lens epithelium and cells transitioning into fiber cells at the lens equator, but absent in differentiated fiber cells. However, at E16.5 in our mutants, CyclinD1 is expressed throughout the entire lens. The cyclin dependent kinase inhibitors (CDKIs) p27kip1 and p57kip2 which work to inhibit cyclinD/cdk activity in post mitotic fiber cells are also misexpressed in the activated smo mutant lens. Beginning at E15.5, p27kip1 and p57kip2, normally expressed in the transitional zone of the lens, display expanded expression in the lens epithelium and entire posterior fiber cell region of the mutants. Furthermore, Proxl and e-mdm, required for proper lens fiber cell elongation and differentiation display ectopic expression throughout the epithelium and fiber cell compartments of the mutants at E15.5 and E18.5.

Conclusions: Constitutive activation of smo promotes the continual activation of a known Hh target gene cyclinD1 which is incorrectly expressed in the fiber cell region of the mutant lenses. The fact that p27kip1 and p57kip2 expression is also misexpressed in the fiber cell population suggests that these CDKIs may be attempting to remove these aberrantly expressing cyclinD1 cells from the cell cycle. Interestingly, Proxl and e-mdm have been shown to regulate p27kip1 and p57kip2 expression, and disruptions in proper expression of these proteins in our mutants may offer insights into the abnormal cell cycle behaviour in our mutant model.

CR: C.L. Kerr, None; J. Huang, None; T. Williams, None; J.A. West-Mays, None
Support: NIH Grant EY11910 (JWM); NIH Grant DE-12728 (TW)

2355 - D794
Comparing the Individual and Combined Roles ofDlg1 and Scrib in the Mouse Lens Epithelium
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Purpose: In Drosophila, studies have shown that PDZ proteins Dlg1 and Scrib are important regulators of cell proliferation, cell adhesion and polarity. Previous work in our laboratory using transgenic and mutant PDZ models has demonstrated a role for PDZ proteins in aspects of cell cycle regulation, polarity and cell adhesion in the lens epithelium. In this study, we extend our knowledge of PDZ function by characterising the lens epithelial phenotypes in mice where Dlg1, Scrib, or both have been deleted.

Methods: Mice carrying Dlg1 or Scrib conditional allele were crossed with MIRL0cre transgenic mice. Double conditional mutants were generated by crossing Dlg1 and Scrib with MIRL0cre (DlgScrb10). Eyes from Dlg1, Scrib, DlgScrb10 and control mice were embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E) to assess the histology of the lens. Immunohistochemistry for Brdu (DNA synthesis marker) was used to examine the cell cycle. Cell adhesion was examined using immunofluorescence with antibodies against cadherin. Sections were immunostained with antibodies against ZO-1, an apical marker, to assess apical-basal cell polarity.

Results: By H&E staining, the appearance of the epithelium in Dlg10, Scrib0 and DlgScrb10 lenses differed from that in control lenses. The epithelium of the Dlg10 lenses appeared thicker than controls, and contained pockets of multilayering and irregularly shaped cells. The Scrib0 epithelium appeared flattened, vacuolated and cells were irregular in shape. The DlgScrb10 epithelium most closely resembled the Scrib10 epithelium. In the Dlg10 cells in Dlg10 lenses was higher than in controls whereas the Scrib0 cells in the Scrib0 lenses did not differ from controls. Interestingly, the Scrib0 cells in the DlgScrb10 lenses was less than controls. In the Dlg10 lenses, E-cadherin staining appeared to be diffused in apical, basal and lateral membranes of centones. However, E-cadherin staining in the Scrib0 lenses appeared not only diffused but also reduced. In controlsZO-1 staining was restricted to the apical surface. In Dlg10, ZO-1 was observed along both the apical and basal membranes of the central epithelium. Both Scrib0 and DlgScrb10 lenses showed loss of ZO-1 from the apical epithelial surface.

Conclusions: These data suggest that both Dlg1 and Scrib are required for maintaining a normal epithelium. However, the effect of each gene individually and together on cell proliferation, cell adhesion and polarity appear to differ. Further studies are in progress to assess the interplay between these genes in the epithelium during lens development.

CR: S. Shutadai, None; A. Griepp, None; R. Rachel, None
Support: E109091, CA88428

2356 - D795
Role of Polycomb Gene Ezh2 in Lens Fibers Differentiation
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Purpose: To investigate the role of Ezh2 in early lens development and during lens fiber differentiation.

Methods: Cre/loxP system was employed to overcome embryonic lethality of Ezh2 KO mice. Lens-Cre mice were crossed with Ezh21/2 mice to achieve conditional inactivation of Ezh2 gene in lens as well as surface ectoderm. Histology and immunohistochemistry has been shown to regulate p27kip1 and p57kip2 expression, and disruptions in proper expression of these proteins in our mutants may offer insights into the abnormal cell cycle behavour in our mutant model.

CR: R. Kuceraova, None; J. Lacheva, None; Z. Koznik, None
Support: None
Integrin-Linked Kinase (ilk) is Critical for Stability of the Lens Capsule

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Purpose: Phosphorylated derivatives of phosphatidylinositol (PI) participate in a number of signaling pathways that can affect broad cellular processes. We wanted to examine the contribution of these phosphoinositides to zebrafish ocular development.

We identified two independent zebrafish cdip (Phosphatidylinositol synthase) mutants that exhibit larval cataracts due to irregular lens epithelial cell proliferation and fiber cell degeneration. The following studies examined the extent and onset of ocular defects within cdip mutants and identified potential signaling pathways that may be affected.

Methods: Histological analysis of PCR-genotyped cdip<sup>-/-</sup> mutants, relative to wild-type siblings, was used to study the onset and the progression of ocular defects within cdip mutants. To identify signaling pathways that may be disrupted in cdip mutant eye tissues, comparative microarray hybridization was performed using RNA extracted from cdip<sup>-/-</sup> mutants and wild-type eyes at 7 dpf. In addition, protein expression was followed in cdip<sup>-/-</sup> mutants and wild-type siblings using immunohistochemistry with antibodies to chick FN1 (Flk1) and CD2, two cellular junction proteins.

Conclusion: None.

Support: This work was supported by NIH Grants EY01739 and EY002162. HB is a recipient of a RPB Career Development Award.

The Role of the Extracellular Matrix in Lens Placode Formation

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Purpose: The mechanism responsible for the formation of the lens placode is not known. We tested the hypothesis, proposed by Hendrix and Zwaan in 1975, that adhesion between the optic vesicle and the surface ectoderm, due to assembly of extracellular matrix (ECM) between them, drives the formation and subsequent invagination of the lens placode.

Methods: Pregnant dams carrying ERT<sup>-/-</sup>, Fn1<sup>cre</sup> or Fn1<sup>cre</sup>/<sup>cre</sup> embryos were injected i.p. with Tamosifen (Tam; 0.2mg/kg) at E8.75 and E9.25. Embryos were collected at E9.5 or E10.25, when the lens placode is formed and invaginating, respectively. Antibody staining for placode-specific molecular markers and with Alexa688-phalloidin was examined by confocal and wide field fluorescence microscopy.

Results: We previously reported (ARVO 2009) that deletion of Pax6 in the surface ectoderm prevented placode formation and decreased the expression of transcripts encoding several components of the ECM, including fibronectin (FN). Since FN is required for assembly of the ECM, we deleted Flk1 globally prior to placode formation using Tam-inducible Cre recombinase. ERT<sup>-/-</sup>, Fn1<sup>cre</sup> embryos treated with Tam were alive, but developed peripheral edema by E10.25, consistent with the known requirement for FN in the formation of heart valves. In these embryos, the progressive placodal ectoderm showed no evidence of thickening and the lens pit did not form on E10.25, although morphogenesis occurred in other regions of the embryo. Placodes and lenses formed normally in Tam-treated, Fn1<sup>cre</sup> embryos that did not carry the ERT<sup>-/-</sup> transgene. Antibody staining confirmed that FN was not detected between the optic vesicle and the ectoderm in the knockout embryos. Staining with antibodies to Pax6 or with phalloidin showed that placode cell specification, cytoskeletal arrangement and cell polarity were normal in these embryos. By labeling the Pax6-expressing surface ectoderm, it appeared that ectoderm cells spread, rather than clustering into the placode. We are analyzing the phenotype of Fn1<sup>cre</sup> embryos generated with two Cre transgenes, targeted to the prospective lens ectoderm and optic vesicle.

Conclusions: Flk1 deficient mouse embryos do not form lens placodes. Failure of placode formation does not appear to result from abnormalities in lens cell specification or polarity. Our data support the "restricted growth model" of Zwaan and Hendrix and suggest that ECM-dependent adhesion between the ectoderm and optic vesicle accounts for placode formation and subsequent lens development.

Support: Research was supported by an unrestricted grant from Research to Prevent Blindness, NIH grant EY04853 and core grant EY02687.

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2610 - A417
Involvement of Calpain in Inonsectin-Induced Cell Death in Cultured Mouse Lens Epithelial Cells
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Purpose: Calpains are calcium-dependent, intracellular, nonlysosomal, cysteine proteases. Activation causes proteolysis of several lens proteins. In rodent lenses, two calpains exist with differing distributions: ubiquitous calpain 2 is highest in lens epithelium, while tissue-preferred calpain 1 is located in cortical and nuclear fibers. Lp2 is a major cause of lens opacities in rodent models. However, only limited information exists concerning the role of calpains in lens epithelium. Thus, the purpose of present experiment was to determine role of calpain 2 in lens epithelial cell death.

Methods: Mouse lens α-TN4 epithelial cells were cultured with calcium ionophore ionomycin to promote calcium influx. Activation of calpains was detected by casein zymography, immunoblotting for calpain fragments, and proteolysis of the calpain substrate o-cresol. LDH release into the culture medium measured cell death.

Results: Calpain 2, but not lower molecular weight calpain 1, were detected in mouse lens epithelial cells. Immunofluorescence induction of LDH from mouse lens epithelial cells, indicating cell death due to calcium influx. Activation of calpain 2 and proteolysis of o-cresol were associated with cell death. Calpain inhibitor SN1945 significantly inhibited the metabolic effects caused by ionomycin.

Conclusions: Ubiquitous calpain 2 was involved in lens epithelial cell death. Lens epithelial cells are important for maintaining lens transparency. Our data suggest that proteolysis by calpain 2 in lens epithelium could contribute to rodent cataract formation.


2610 - A418
Correlation of Loxl1 Expression in Lens Epithelial Cells With Clinical Signs of Pseudoexfoliation
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Purpose: Pseudoexfoliative (PXE) syndrome is a major risk factor for glaucoma and affected individuals have a higher rate of complications during cataract surgery. PEX material is mainly produced by the epithelial cells of the iris, lens and cilary body. Variants of the lysyl oxidase-like 1 (LOXL1) - a gene involved in cross-linking elastin - are strongly associated with PEX syndrome. We aimed to investigate the immunohistochemical expression of LOXL1 in lens capsule specimens of affected individuals.

Methods: Anterior lens capsule specimens were collected from routine phacoemulsification cataract surgeries. Patients with PEX (+<15, mean age <79.7 ± 7.2) and normal controls (+<24, mean age >72.94 ± 5.59) were included in this study. PEX patients were determined by clinical slit-lamp examination. Paraffin-embedded sections from all thirty-nine anterior lens capsulcs were immunostained with mouse-anti-human LOXL1 polyclonal antibody (1:100. Abcam Inc., Cambridge, MA, USA) using the Ventana Benchmark fully automated machine.

Results: In the PEX group, 15 of 15 (100%) cases demonstrated immunopositivity for LOXL1 in the lens epithelial cells, while in the control group, only 1 of 24 (4%) cases were positive (p<0.0001). PEX material could be identified by light microscopy on the basis of compact hyaline rim around the surface of the capsule in 7 of 15 (47%) capsules in the PEX group, of which all were positive for LOXL1.

Conclusions: The immunohistochemical approach in this report has led to the identification of LOXL1 as a major component of PEX fibers and is consistent with previous reports. The significant correlation between the expression of LOXL1 in epithelial lens cells and clinical PEX may indicate the involvement of LOXL1 in the formation of PEX material. Our results warrant further studies investigating the role of LOXL1 in early stages of PEX and possible therapeutic implications.

CR: D. Faingol’d, None; O. Kasner, None; E. Antecka, None; B.F. Fernandes, None; M. Greenberg, None; M.N. Burnier, None. Support: None

2612 - A419
LEDGF Expression in Human Lens Epithelial Cells is Transcriptionally Regulated by the SP1 Response Elements
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Purpose: The transcription factor LEDGF fills its pro-survival, cytotoxic protective function by upregulating the transcription of small heat shock protein (shsp) genes. LEDGF is enriched in human and mice lens epithelial cells (LECs), but the factor’s regulation is largely unknown. We investigated the key transcription factor, SP1, and its cis-acting elements, GC box(es), controlling/regulated LEDGF expression in human LECs.

Methods: A Web-based computer analysis (MatInspector, Genomatix) was used to spot out putative transcription factor binding sites. 5’-flanking sequence spanning from -1269 to -35 bps of LEDGF gene was subcloned into pCAT-basic vector. Deletion mutants -1007, -316, -175, -132, -65 and -35 were prepared by PCR and cloned into p-CAT-basic vector with common 3’ end (+35 bps). Gel-shift and supershift assays, mutational analysis and CAT assay were used to characterize transcriptional protein(s) that physically and functionally bind to DNA element(s) of LEDGF promoter. pCMV-SP1 transfection studies demonstrated that SP1 is an SP1 transactivator. Western blot and real-time PCR were used to monitor expression levels of transcriptional protein(s) and LEDGF in LECs.

Results: Bioinformatic analysis of LEDGF promoter disclosed that LEDGF promoter near the transcription start site was devoid of TATA and CAAT boxes, but bore a heat shock gene motif enriched in human and mice lens epithelial cells, but the factor(s) regulating LEDGF expression was largely unknown. LEDGF promoter activity was decreased in SP1−/− LECs, while tissue-preferred Lp82 is located in cortical and nuclear fibers. Lp82 and 43 of HSP27/α B-crystallin genes and in protecting lens epithelial cells (LECs) facing stress.

Conclusions: Findings provide first insight into the transcriptional regulation of LEDGF expression by SP1 response elements, and may provide clues to controlling LEDGF expression, which is essential for cellular survival.

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2613 - A420
LEDGF and HSF1 Interaction is Essential in Transactivation of Heat Shock Protein Genes in Lens Epithelial Cell Survival During Stress
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Purpose: In normal physiological conditions, the nuclear protein LEDGF binds to stress response (STRE; A/TGGGGA/T) and heat shock (HSE; nGAAn) elements in the small heat shock protein (shsp) genes and protects cells by activating those genes. Under exogenous stress (HTS), LEDGF interacts with trimeric form of HSF1 to activate the heat shock genes and thereby protecting lens epithelial cells from heat stress.

Methods: Using a double-standard DNA oligomer with HSE and STRE (A/TGGGGA/T) elements (nGAAn) and its repeats and their mutants in gel-shift assay coupled with transcriptional activity was measured by CAT-ELISA at 37°C. The interaction between LEDGF and HSF1 during heat stress was done by formation of PEX material. Our results warrant further studies investigating the role of Loxl1 in early stages of PEX and possible therapeutic implications.

Conclusions: This study has led to the identification of Loxl1 as a major component of PEX fibers and is consistent with previous reports. The significant correlation between the expression of LOXL1 in epithelial lens cells and clinical PEX may indicate the involvement of LOXL1 in the formation of PEX material. Our results warrant further studies investigating the role of LOXL1 in early stages of PEX and possible therapeutic implications.

CR: D. Faingol’d, None; O. Kasner, None; E. Antecka, None; B.F. Fernandes, None; M. Greenberg, None; M.N. Burnier, None. Support: None

2614 - A443
Correlation of Loxl1 Expression in Lens Epithelial Cells With Clinical Signs of Pseudoexfoliation
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Purpose: Pseudoexfoliative (PXE) syndrome is a major risk factor for glaucoma and affected individuals have a higher rate of complications during cataract surgery. PEX material is mainly produced by the epithelial cells of the iris, lens and ciliary body. Variants of the lysyl oxidase-like 1 (LOXL1) - a gene involved in cross-linking elastin - are strongly associated with PEX syndrome. We aimed to investigate the immunohistochemical expression of LOXL1 in lens capsule specimens of affected individuals.

Methods: Anterior lens capsule specimens were collected from routine phacoemulsification cataract surgeries. Patients with PEX (+<15, mean age <79.7 ± 7.2) and normal controls (+<24, mean age >72.94 ± 5.59) were included in this study. PEX patients were determined by clinical slit-lamp examination. Paraffin-embedded sections from all thirty-nine anterior lens capsulcs were immunostained with mouse-anti-human LOXL1 polyclonal antibody (1:100. Abcam Inc., Cambridge, MA, USA) using the Ventana Benchmark fully automated machine.

Results: In the PEX group, 15 of 15 (100%) cases demonstrated immunopositivity for LOXL1 in the lens epithelial cells, while in the control group, only 1 of 24 (4%) cases were positive (p<0.0001). PEX material could be identified by light microscopy on the basis of compact hyaline rim around the surface of the capsule in 7 of 15 (47%) capsules in the PEX group, of which all were positive for LOXL1.

Conclusions: The immunohistochemical approach in this report has led to the identification of LOXL1 as a major component of PEX fibers and is consistent with previous reports. The significant correlation between the expression of LOXL1 in epithelial lens cells and clinical PEX may indicate the involvement of LOXL1 in the formation of PEX material. Our results warrant further studies investigating the role of LOXL1 in early stages of PEX and possible therapeutic implications.

CR: D. Faingol’d, None; O. Kasner, None; E. Antecka, None; B.F. Fernandes, None; M. Greenberg, None; M.N. Burnier, None. Support: None
2614 - A424
Sumoylation of the 32 kD Pax-6 Activates Its Transcriptional Activity
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Purpose: Pax-6 is a master regulator controlling eye and brain development. Four isoforms of Pax-6, p46, p48, p43, and p32/33, have been detected in the vertebrate eye. Absence of the paired domain in the 32 kD-Pax-6 differentiates it from the other three forms. How is the 32 kD-Pax-6 acting as a transcription factor remains unknown. In the present study, we demonstrated that activation of the 32 kD-Pax-6 requires sumoylation, an important posttranslational modification.
Methods: Western-blot analysis was used to detect the nuclear and cytoplasmic distributions of Pax-6 in human lens epithelial cell line (FHL-124). Gel mobility-shifting assays were used to test the binding activities of different forms of Pax-6 to a P3 sequence specific for homedomain. In vitro sumoylation assay was conducted to detect sumoylation of 32 kD-Pax-6. Immunohistochemistry and co-immunoprecipitation were used to detect the in vivo interactions between Pax-6 and sumo proteins.
Results: Three Pax-6 isoforms (46-, 43-, and 32-kD) were detected in the nuclear extracts but only one isoform (43-kD) was found in the cytoplasm. Only the 43-kD Pax-6 displayed binding ability to the P3 sequence. The 32- and 46-kD Pax-6 synthesized in vitro lack the binding ability to P3 sequence. After incubating with the Pax-6-depleted nuclear extract, the 32-kD and not the 46-kD Pax-6 exhibited strong binding ability to P3 sequence. This binding activity of 32-kD Pax-6 was prevented when the nuclear extract was pre-cleared with sumo antibodies. The interacting complexes of Pax-6 and sumo proteins were detected in developing mouse eye.
Conclusions: The 32-kD and 46-kD Pax-6 display differential DNA binding activities to the P3 sequence and likely regulate different downstream target genes. Moreover, sumoylation of the 32-kD Pax-6 is necessary to activate its transcriptional activity.
CR: L. Gong, None; Q. Yan, None; D. Yuan, None; L. Zhang, None; J.-P. Liu, None; M. Deng, None; S. Sun, None; H. Ma, None; D.W. Li, None.
Support: NIH Grant EY15765 and EY18380

2615 - A422
Cell Damage and Expression of Cytokines in Human Lens Epithelial Cells Following Exposure to Prostaglandin Analogs and Their Preservatives
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Purpose: To investigate cell damage and the formation of cytokines in a human lens epithelial (HLE) cell line (SRAD1/04) exposed to either latanoprost (LP), travoprost (TP), benzalkonium chloride (BAC) or a new preservative, sozila (SOF).
Methods: HLE cells were cultured in DMEM with 5% FBS. The topical drop concentration of LP (50µg/ml), TP (40µg/ml) or BAC (200µg/ml) or 2X dilution of SOF, and their 10X to 1000X dilutions were added to the medium. Cell morphology was observed by phase-contrast microscopy. Each exposure medium was collected following 7 days of culture for the analysis of PGE2, IL1 and IL6.
Results: With a 10X dilution of SOF, no proliferation or elongation of the cells was observed. There were no remarkable changes in cell morphology and the release of cytokines with the 100X and 1000X dilution of SOF. At the 100X dilution, the ratio of cytokine release compared to control was 0.5-1.8 for LP, TP and SOF. At the 100X dilution of BAC, none of the cells survived and even at the 1000X dilution of BAC, the release of cytokines was significantly greater than control (P < 0.05). In contrast, the release of cytokines by 10X dilution of LP or TP were 1to 7X higher than those of the control.
Conclusions: We have previously reported eye drops for glaucoma cause cytokoid macular edema after cataract surgery, suggesting the increase of cytokine release by the preservative, BAC, in HLE cells. These new results indicate that while all components induce some level of cell damage and formation of cytokines in HLE cells, BAC is the most toxic.
CR: N. Ibaraki, None; K. Miyake, None.
Support: None

2616 - A423
Migration of Human Lens Epithelial Cells on the Vertical Wall Through Square Edge of Culture Dish
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Purpose: To evaluate human lens epithelial (HLE) cells can migrate on the vertical wall through square edge of culture dish.
Methods: HLE cell line (SRAD1/04) cells were cultured on the 25cm² culture flask. To study the ability to migrate on the vertical wall through square edge, the cells on the vertical wall were observed by phase-contrast microscopy. To evaluate the growing rate on the vertical wall, the following procedures were studied. After reaching the confluent, the cells were scraped off only a little area remaining. And then, the culture medium was filled and the flask was stood for the flask bottom became on the vertical position. The cell growing rate was measured on the up-growth group and the down-growth group.
Results: The cells could migrate on the vertical wall from the bottom of the flask. The square edge of culture flask was confirmed by scanning microscopy. At 3rd day of culture, the growing edge of up-growth and down-growth group reached 0.84 and 1.17 mm (p<0.005), respectively.
Conclusions: HLE cells migrate not only the bottom of the culture dish but also on the vertical wall. Only the square edge of the matrix does not prevent the posterior capsule opacification (PCO). The prevention of PCO by square edge of IOL may be affected by other factor from the square edge.
CR: I. Yoshioka, None; N. Ibaraki, None.
Support: None

2617 - A424
Calpain and Caspase-12 Activation Mediates Apoptosis in Transgenic Mouse Lens Expressing a Dominant-Negative Mutant of FGFR
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Purpose: To express a dominant negative (DN) mutant of FGFR, which is a truncated form of FGFR1, in the transgenic mouse lens can induce cell death in the lens fiber cells. Previously we demonstrated that DN-FGFR-induced cell death is independent of the classical mitochondrial apoptosis mechanism. The alternative cell death mechanism is investigated in this study.
Methods: Water-soluble proteins were prepared from newborn wild type (WT) and DN-FGFR mouse lenses. Calpain activation was evaluated by the presence of cleaved crystalline fragments. Cleaved (active) caspase-12 in the lens homogenate was detected by western blot. Caspase-12 localization in mouse lens was demonstrated by immunofluorescence.
Results: Alpha- and beta-crystallin breakdown products were detected by western blot in the DN-FGFR lenses. The same crystallin proteolytic patterns were seen in the WT lens proteins after incubation with 1 mM calcium at 37°C for one hour. These results suggested that abnormal activation of calpain occurred in the DN-FGFR lens. A low level of cleaved caspase-12 was present in the WT lens. In comparison, the level was significantly increased in the DN-FGFR transgenic lens. Immunostaining showed that, in the WT lens, caspase-12 was found in the lens epithelial cells. During fiber cell differentiation, caspase-12 was localized to the nuclei of the fiber cells. In contrast to the WT lens, caspase-12 immunofluorescence in the DN-FGFR lens did not co-localize with the cell nuclear staining, suggesting that caspase-12 released from the cell nuclei can be cleaved (activated) by the calpain in the cytoplasm in the presence of calcium.
Conclusions: We have identified a new calpain activated caspase-12 mediated apoptosis pathway in the lens fiber cells. We propose that accumulation of folding- incompetent DN-FGFR proteins in the lens fiber cells can induce ER stress, disrupt calcium homeostasis, and result in the activation of calpain and caspase-12 which contributes to the mechanism of apoptosis.
CR: L.W. Reneker, None; H. Chen, None.
Support: NIH Grants EY13146 and EY14795, and Research to Prevent Blindness (RPB)
Organizing Section: LE

2618 - A425
Interferon-Gamma Induces Indoleamine 2,3-Dioxygenase and Causes Apoptosis in Lens Epithelial Cells
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Purpose: We investigated the effects of interferon-γ (IFN-γ) on IDO-mediated synthesis of kynurenines and on apoptosis in human lens epithelial cells (HLE-B3).

Methods: HLE-B3 cells were cultured in the presence of 1-100 units of IFN-γ for 2 days. The effects on IDO expression and its downstream signaling pathways were investigated using an enzyme activity assay and Western blotting. Intracellular production of kynurenines was assessed by HPLC. Apoptosis was determined by Hoechst staining.

Results: IFN-γ promoted the synthesis of IDO and activated the JAK/STAT signaling pathway in HLE-B3 cells in a dose-dependent manner. Fludarabine, a STAT1-specific inhibitor, blocked IFN-γ-mediated IDO expression. NFK, KYN and 3OHKyn were detected in cells, with 3OHKyn concentrations 2-3 fold higher than other kynurenines. The intracellular production of kynurenines could be completely inhibited by 1-methyl-D,L-tryptophan (MT), an inhibitor of IDO. All three kynurenines were detected in the cell culture medium from IFN-γ-treated cells. Kyn- and 3OHKyn-modified protein were detected in IFN-γ-treated cells. Induction of IDO by IFN-γ caused elevations in hydrogen peroxide levels, caspase-3 activity and apoptosis in HLE-B3 cells. At equilibrium concentrations, 3OHKyn caused higher levels of apoptosis than the other kynurenines in HLE-B3 cells. MT and a kynurenine 3-hydroxylase inhibitor (RO61-8041) effectively inhibited IFN-γ-mediated apoptosis in HLE-B3 cells.

Conclusions: Our results show that induction of IDO by IFN-γ is JAK1/2-STAT1 pathway-dependent, and that this induction causes 3OHKyn-mediated apoptosis in HLE-B3 cells. Our study provides evidence that IDO-mediated kynurenine formation can play a role in cataract formation related to chronic inflammation.

CR: R. H. Nagaraj. None; M. Mailankot. None.
Support: NIH grants R01EY01629, R01EY-09912, P350-E11537, RPB and OMERF

2620 - A427
The Roles of Plasminogen Activator Inhibitor-1 for the Pathogenesis of Atopic Cataracts
K. Horic, A. Matsuda, K. Imai, K. Morii, J. Hammar, S. Kimohiye, A. Murakami. Department of Ophthalmology, Juntendo University School of Medicine, Tokyo, Japan; Department of Ophthalmology, Kyoto Prefectural Univ of Med, Kyoto, Japan; Department of Ophthalmology, Juntendo University School of Medicine, Tokyo, Japan.

Purpose: We previously showed an association between interferon-γ (IFN-γ) receptor polymorphisms and occurrence of atopic cataracts (IOVS 48:583-589, 2007). A recent study showed that IFN-γ is essential for PAI-1-induced post-operative fibrosis in the lens (Nat Med. 14:437-441, 2008). In this study we further tried to clarify the roles of a fibrosis related protein, Plasminogen Activator Inhibitor-1 (PAI-1), for the pathogenesis of atopic cataracts.

Methods: Real-time-PCR analysis was carried out to compare the PAI-1 mRNA expression between the cDNA samples obtained during cataract surgery from the lens epithelium of atopic cataracts (n=10) and of senile cataracts(n=10). Cultured lens epithelial cells were stimulated by recombinant IFN-γ, and compared PAI-1 expression by real time PCR and Western blotting. Immunohistochemical analysis was carried out using the antiserum capsular tissue obtained from atopic cataracts.

Results: The relative expression of PAI-1 mRNA was significantly higher in the cDNA samples obtained from atopic cataracts compared to those from senile cataracts. Recombinant IFN-γ stimulation induced 4.6-fold higher PAI-1 mRNA expression in cultured lens epithelial cells at 24 hours after stimulation. PAI-1 positive immunostaining was observed in the subcapsular fibrosis of atopic cataracts.

Conclusions: Our results suggested that IFN-γ-PAI-1 signaling cascade plays some roles for the pathophysiology of atopic cataracts.

CR: K. Horic, None; A. Matsuda, None; K. Imai, None; K. Morii, None; J. Hammar, None; S. Kinohiye, None; A. Murakami, None.
Support: None

2619 - A426
Characterization and Functional Expression of the Natriuretic Peptide System in Human Lens Epithelial Cells
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Purpose: The family of natriuretic peptides (NPs) consists of three peptides; atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) (Jpn J Pharmacol 72:245-253, 1997). As well as three allied receptors (NPsR); natriuretic peptide receptor A (NPR-A), natriuretic peptide receptor B (NPR-B) and natriuretic peptide receptor C (NPR-C). We demonstrate both expression and functionality of the natriuretic peptide system (NPS) in the human lens epithelial cell line, HLE-B3, and suggest their potential role in protection against mitochondrial permeability transition (MPT).

Methods: RT-PCR coupled with confirmation by DNA sequencing was used to demonstrate expression of mRNA for the NPS. Protein expression and subcellular localization of the natriuretic peptide receptors was determined by utilizing formaldehyde-fixed, Saponin-permeabilized cells using conventional immunofluorescence techniques. Functionality was illustrated by a cyclic GMP (cGMP) response to NPs using an enzyme-linked immunosorbent assay.

Results: NPS, a mediator of guanylyl cyclase activation, and a crucial player in the PI3/Akt pathway of protection against MPT, was undetectable in cultured HLE-B3, as confirmed by Western blot analysis and comparison with an eNOS standard. HLE-B3 cells express mRNA for ANP, BNP and CNP along with their associated receptors. Confocal microscopy and immunofluorescence indicated diffuse cytosolic localization for all three NPsRs; immunostaining being greater for NPR-A and NPR-B when compared to NPR-C. All three NPsR s elude a cGMP response in the rank order CNP>>ANP=BNP.

Conclusions: The natriuretic peptide system is expressed and operative in cultured human lens epithelial cells. Classically, the NPS is involved in the regulation of physiological functions; including blood pressure and fluid homeostasis, and for ocular systems, to influence eye pressure. We suggest a second, novel role for the NPs in HLE-B3 cells. In the event of low or missing eNOS activity, the NPs and NPsRs additionally function as local autocrine/paracrine mediators, which under appropriate conditions may substitute for eNOS in the PI3/Akt pathway of mitochondrial protection, activating guanylyl cyclase and resulting in a cGMP response, stimulating downstream mitoK<sub>ATP</sub> channels, abrogating mitochondrial permeability transition and otherwise ensuing cell death.

CR: P. R. Cammarata, None; B. Braun, None; J. Pack, None.
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2621 - A428
Conditional Deletion of Notch2 in Lens
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Purpose: Notch signaling is highly conserved and regulates transcription during diverse developmental and physiological processes. Key players of canonical notch signaling are Notch receptors (I-4), ligands - Jagged (1,2) and Delta (1,3,4) and CSL (Cbp/Pan/COM) and RBP-J (IFN-γR1 or RBP) in mammalian family transcription factors. Notch intracellular domain (NICD) is released upon ligand binding to the Notch receptor and represents the activated Notch, which translocates to the nucleus and forms a transcriptional complex with CSL to initiate transcription of target genes. Previous studies using conditional gene targeting in mice have shown that disruption of Notch signaling in lens by ablation of RBP-J or Jagged1, led to a block in proliferation of the lens epithelium, leading to aberrant expression of cell cycle regulatory genes, premature differentiation, and a dysgenic lens. Our own previous studies demonstrated a role for Jagged2 in lens, but this study will evaluate the role of Notch2 signaling in secondary cell differentiation establishing distinct dual roles for canonical Notch signaling in the lens proliferation and differentiation. However, relative contributions of the two Notch receptors Notch1 and 2 expressed in the lens, is still unclear. This work investigates Notch2 receptor mediated signaling in lens differentiation and development by generating and characterizing the ocular phenotypes of lens specific conditional knockouts of Notch2 receptor (Notch2CN) in mice.

Methods: Notch2CN mice were generated using Cre-Lox approach by crossing mice carrying Le-Cre driver (Ashrey-Padan et al, 2000) and Notch2 flox/flox (McCright et al, 2006). The ocular phenotypes were analyzed using histology.

Results: Notch2CN mice were microphthalmic, lenses lacked epithelial cells and fiber cells were disorganized and failed to elongate normally. Cataract was of common occurrence in Notch2CN mice as was the fusion of lens tissue with iris. Papillary opening was smaller or non-existent in some Notch2 CN.

Conclusions: Notch2 receptor mediated signaling is critical for canonical Notch signaling in the lens. The severity of the lens defects in the Notch2CN mice appeared to be greater than those of the RBP-JCN and that of Notch1CN mice and comparable to that of Jag1 mice.

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Lens capsules from a variety of different mammals, including human, were obtained during cataract surgery (n=48). Consecutive samples were immediately analyzed by the comet assay, others fixed and processed for immunohistochemistry or TEM. A third group was cultured in DMEM-F12 with 15% FBS for 2 weeks prior to examination by light microscopy and TEM.

Results: In fresh lens epithelial cells we observed relatively high levels of formamidopyrimidine DNA glycosylase (FPG) -sensitive sites compared with endonuclease III - sensitive sites. Generally there were very low levels of strand breaks. Immunohistochemical staining for PCNA was prominently positive whereas Ki67, Caspase 3 and TUNEL were negative. Light microscopy and TEM of cultured epithelium showed a basically retained morphology.

Conclusions: For the first time we demonstrate the DNA damage profile using the comet assay in human lens epithelial cells from patients with cataract. The very low level of strand breaks reflect the excellent quality and ease of handling of these cells. Most striking are high levels of FPG-sensitive sites compared with EndoIII-sensitive sites, which might indicate a preparodernation of damage caused by single oxygen. We tested the possible mode of action light in the presence of a variety of photosensitisers, which leads to 8-oxoguanine as the predominant product. Our results further indicate a high level of DNA repair, a neglectable level of apoptotic activity, and the usefulness of the ex vivo system in assays aimed at exploring the activity of biosubstances in prophylaxis of DNA damage and as effectors of DNA repair.

CR: O. Ringen, None; A. Oscoz, None; B. Nicolaissen, None; CR: P.A. Ruzycki, None; G.J. Zablocki, None; S. Palla, None; D.A. Ammar, None; B.G. Reddy, None; J.M. Petras, None; Support: NIHR Grant EY05856 (UCDenver); DST (Hyderabad).

2623 - A430
Anterior Plaque Formation Phenotype Linked to Activation of Aldose Reductase (AKR1B1)


Purpose: Transgenic mice that express high levels of human aldose reductase (AKR1B1) develop an unusual phenotype consistent with aberrant differentiation of lens epithelial cells. The purpose of this study was to characterize this phenotype and to probe the role of activated aldose reductase in mediating abnormal differentiation of epithelial cells.

Methods: Lenses were obtained from AKR1B1 transgenic mouse strains and nontransgenic controls. Immunofluorescence and H&E staining were used to characterize and evaluate lens phenotype. To determine a role of AKR1B1 in the degenerative phenotype, transgenic and non-transgenic control litters were treated with sorbinil, an aldose reductase inhibitor (ARI). Sorbinil was provided to the mother and pups from P1 in the drinking water and lenses evaluated at P14 and 5 weeks.

Results: AKR1B1 transgenic mice with the highest levels of protein expression (PAR37 and PAR40) display a lens degenerative phenotype while the strain with much lower expression (PAR40) exhibits normal lens morphology. The degenerative phenotype involves lens opacity, the abnormal localization of nucleated cells both beneath the anterior epithelium as well as near the posterior pole and the presence of large vacuoles primarily in the lens bow region. Abrupt nucleated cells were present between the cortical fibers and a sheet of plaques extending from the anterior epithelium. The plaques are densely packed and positively stain for α-smooth muscle actin, a marker for epithelial-to-mesenchymal transition (EMT). The phenotype was suppressed through treatment with sorbinil. Treated high expressing transgenic mice had reduced lens opacity and exhibited normal nuclear localization while only vacuoles in the lens bow region remained.

Conclusion: Elevated levels of AKR1B1 expression may have consequences not only through metabolic product accumulation but also through effects on lens cell differentiation, localization, and cell cycle kinetics.

CR: P.A. Ruzyczki, None; G.J. Zabocki, None; S. Palla, None; D.A. Ammar, None; B.G. Reddy, None; J.M. Petras, None; Support: NIHR Grant EY05856 (UCDenver); DST (Hyderabad).

2624 - A431
Selective Up-Regulation of MAPK Signaling by Aldose Reductase (AKR1B1)

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Purpose: Patients with diabetes have an increased incidence of blindness that may be linked to hyperglycaemia. Activation of the poyol pathway due to increased aldose reductase activity is one of the several mechanisms thought to be associated to lens opacity, but the underlying mechanism remains ambiguous. Here we used human aldose reductase (AKR1B1) and human small intestine aldose reductase (AKR1B10) transgenic mice to investigate how AKR1B1 might influence MAPK activity. We also examined the MAPK signaling pathway stimulated by hyperglycemic conditions in vitro.

Methods: Lenses from AKR1B1 and AKR1B10 transgenic mice, together with nontransgenic controls for the repair of lens plaques (AKR1B10 transgenic mice only) by Western blotting (WB). In lenses, phosphorylation of MAPKs proteins was measured by WB after AKR1B1 inhibitor treatment of above transgenic mice. Effects of hyperglycemia in respect to MAPK proteins were evaluated by WB following high glucose culture of human lens epithelial B3 (HELEB3) cells with or without aldose reductase and MEK/ERK inhibitors.

Results: Strong activation (~10-fold) of ERK1/2 MAPK protein was observed only in AKR1B1 mice but not in AKR1B10 or nontransgenic controls. In transgenic mice, a moderate increase in AKR1B1 is sufficient to induce MAPK activation; however, further elevation (3-fold) of expression levels of AKR1B1 do not further increase activation. This activation effect was attenuated (~60%) by sorbinil, an AKR1B1 inhibitor in both transgenic lenses and HLEB3 cells exposed to 25.5mM glucose. Moreover, high glucose significantly increased the fold) the AKR1B1 level in a time-dependent manner and induced ERK1/2 activation. Meanwhile, supplementation of PD98059 and U0126, the specific inhibitors of MAPK/ERK kinase, reversed the activation of ERK1/2 (70-90%) under high glucose in HLEB3 cells.

Conclusions: These results indicate that AKR1B1 plays an important role in the regulation of the ERK1/2 MAPK phosphorylation and high glucose can increase AKR1B1 and ERK1/2 MAPK activity in HLEB3 cells, thereby providing further insight into the molecular mechanism of diabetic eye disease.

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2625 - A432
Cell Organisation in the Mammalian Lens Epithelium


Purpose: To measure the cell complement of the mammalian lens epithelium and to correlate cell number, organisation and geometry relative to their location in order to understand the biological basis of lens size.

Methods: Lens capsules from a variety of different mammals, including human, were flat mounted and processed for confocal immunofluorescence microscopy. Image processing software was developed for the rapid and reliable counting of cells within these epithelial samples. Mouse, rat, bovine and human lens epithelia were studied. Rates of apoptosis and cell proliferation were determined by TUNEL labelling and Ki67 staining respectively. Cell boundaries were imaged using either phallolidin or lectin conjugates to allow volume and individual dimensions to be measured.

Results: Using both individual cell parameters as well as cell number measurements, the cell complement of the mammalian lens epithelium and also the usefulness of the ex vivo system in assays aimed at exploring the activity of biosubstances in prophylaxis of DNA damage and as effectors of DNA repair.

CR: O. Ringen, None; A. Oscoz, None; B. Nicolaissen, None; CR: P.A. Ruzyczki, None; G.J. Zabocki, None; S. Palla, None; D.A. Ammar, None; B.G. Reddy, None; J.M. Petras, None; Support: NIHR Grant EY05856 (UCDenver); DST (Hyderabad).
**2626 - A433**
The Effect of NF-κB Inhibiting siRNA on Posterior Capsular Opacification

**Purpose:** The aim of this study was to investigate the effect of small interfering RNA (siRNA) of nuclear factor kappa B (NF-κB) on the development of posterior capsule opacification (PCO) in in vitro and in vivo study with rabbits.

**Methods:** After application of p105 NF-κB siRNA to lens epithelial cells (LECs), western blot analysis to detect p105 and p50 NF-κB and scratch assay was done. In capsular bag model, LEC count was significantly decreased and immunocytochemistry showed decrease of p50 NF-κB on the posterior capsule.

**Results:** By applying p105 NF-κB siRNA to LECs, p105 NF-κB and p50 NF-κB were decreased by western blot analysis and migration of LECs was inhibited by scratch assay. In capsular bag model, cell count was significantly decreased and immunocytochemistry showed decrease of p50 NF-κB on the posterior capsule. In the in vitro study with rabbits, p105 NF-κB siRNA effectively decreased PCO with both slit-lamp examination and POCOMan software assessment.

**Conclusions:** NF-κB seems to be related to the migration and proliferation of LECs. And using NF-κB siRNA shows to be effective in inhibiting the migration and proliferation of LECs in vitro and decreased PCO formation after cataract surgery in a rabbit.

**CR:** C.-K. Joo, None; H.-Y. Park, None; K.-M. Lee, None; M.-O. Park, None; J.-S. Choi, None.

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**2627 - A434**
Protein Phosphatase-1 Acts as a Major Phosphatase in the Ocular Lens and Regulates Multiple Important Targets Including P53, Pax-6 and Akt
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**Purpose:** Protein serine/threonine phosphatase-1 (PP-1) is a key phosphatase in the ocular lens and regulates development, differentiation and pathogenesis. We have recently demonstrated that PP-1 plays an important role in regulating the functions of the tumor suppressor, p53, and the eye development regulator, Pax-6. In the present study, we present evidence to show that PP-1 is the major phosphatase regulating AKT signaling pathway.

**Methods:** Dephosphorylation assay was used to identify possible phosphatase. Co-immunoprecipitation and immunocytochemistry were used to determine the interaction between PP-1 and AKT. SHRNA and overexpression were used to knock down or overexpress PP-1 or PP-1β by siRNA leads to enhanced phosphorylation of AKT at Thr-450. Overexpression of PP-1 or PP-1β results in attenuated phosphorylation of AKT at Thr-450. Moreover, our results demonstrate that PP-1 significantly modulates AKT functions in regulating expression of the downstream genes coding for NF-κB, GS-K3, α- and β-crystallins, and N-cadherin, promoting cell survival and modulating differentiation.

**Conclusions:** Our results demonstrate that PP-1 acts as a major phosphatase to dephosphorylate AKT at Thr-450 and thus modulate functions of AKT signaling pathway. In addition, PP-1 is a major phosphatase in the ocular lens and regulates functions of multiple targets including p53, Pax-6 and AKT.

**CR:** D.W. Li, None; L. Xiao, None; L. Gong, None; D. Yuan, None; M. Deng, None; L. Zhang, None; J. Liu, None; S. Sun, None; J. Liu, None; H. Ma, None.

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**2628 - A435**
Abnormal Fiber End Migration Leads to Posterior Subcapsular Cataract (PSC) Formation in Royal College of Surgeons (RCS) Rats
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**Purpose:** Evaluation of posterior fiber ends of RCS rat lenses reveals distinct structural and cytoskeletal changes as early as 3wks post natal that lead to PSC formation. These include multiple suture sub-branches, altered fiber end morphology and associated BMC changes. The objective of this study was to detail fiber end changes that precede and culminate in a PSC in RCS rats.

**Methods:** A total of 35 RCS rats (2-6wk old), were euthanized, lenses removed and photographed. All OD lenses were processed for laser scanning confocal microscopy (LSCM) to localize BMC components, and all OS lenses for scanning electron microscopy (SEM) to evaluate fiber end morphology.

**Results:** At 2wks, lenses displayed the typical inverted-Y suture pattern on the posterior, and by 3wks most lenses had at least one sub-branch. Additional sub-branches were observed with time, opacities being visible as early as 4wks and progressing into PSC plaques by 6wks. SEM revealed fiber ends with normal size, shape, arrangement and filopodia at 2wks, scattered areas of dome-shaped fiber ends and small filopodia at 3wks, and irregularly arranged dome-shaped ends at 4wks. Filopodia at 4wks were extremely long and had ‘boutons’ at their tips. Extensive membrane blebbing and cell separation were also seen at this age. LSCM showed normal distribution of cadherin, vinculin and β1 integrin at 2wks. F-actin although normal at 2wks, was visible as scattered bright foci at 3wks, which increased in incidence by 4wks. The BMC at 2wks was distinct with F-actin rearranged as a ‘rosette’ pattern with bright foci at the vertices. At all ages, cadherin delineated the fiber ends and integrin was arranged as plaques within the ends throughout the BMC. Integron labeling was present at both peri-sutural and sutural regions, which is contrary to that seen in normal rat lenses.

**Conclusions:** The initial changes to fiber ends (2-4wks) are accompanied by minor changes to the BMC indicating that migration of the ends continues, albeit misdirected, resulting in abnormal suture sub-branches. Between 4-6 wks, the changes are more extreme and are accompanied by distinct rearrangements of normal BMC architecture. The data suggest that a modification of adhesion mechanics at the BMC, specifically, cell-cell contact, matrix-cell adhesion and tissue fiber end detachment, are consistent with a cessation of fiber end migration in RCS rat lenses.

**CR:** A. Joy, None; K.J. Al-Ghoul, None.

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**2629 - A436**
The P53 Target Gene, Bak, Is Involved in Mouse Lens Differentiation
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**Purpose:** The mitochondrial cell death pathway plays a critical role in mammalian lens differentiation. Our previous studies showed that the tumor suppressor, p53, regulates bFGF-induced lens fiber cell differentiation through control of the Bcl-2 family member, Bak. However, how Bak mediates regulation of lens differentiation remains unknown.

**Methods:** The basic FGF-induced lens fiber differentiation model was established using cultured mouse lens epithelial cell line, OTN4-1. Stable clones of p53 and bak knockdown were generated through respective overexpression of the p53 and bak shRNA plasmids. The FGF induction of lens differentiation in the normal or knockdown or overexpression of either p53 or Bak was analyzed by cell fraction, confocal microscopy and co-immunoprecipitation.

**Results:** Phosphorylation of p53 at Ser-15 and Ser-20 was enhanced, and Bak expression was also up-regulated during FGF-induced lens differentiation. Moreover, the mouse lens epithelial cells were induced to form the lentoids and express the differentiation marker, β-crystallin. However, in p53-knockdown lens epithelial cells, phosphorylation of p53 and expression of Bak were significantly attenuated. Associated with this attenuation, the mouse lens epithelial cells remained undifferentiated. In Bak-knockdown lens epithelial cells, similar phenotype was also observed. Furthermore, during bFGF induction, phosphorylated p53 at Ser-15 was accumulated in cytoplasm. The cytoplasmic p53 was found interacting with Bak. In addition, beside the strong expression of Bak in cytosol, it was also found in nuclear fraction and the nuclear faction was increased during FGF induction.

**Conclusions:** Our results suggest that Bak, the pro-apoptotic member of the Bcl-2 family mediates p53-dependent bFGF-induced lens differentiation.

**CR:** M. Deng, None; L. Xiao, None; L. Gong, None; D. Yuan, None; M. Deng, None; L. Zhang, None; J. Liu, None; S. Sun, None; J. Liu, None; H. Ma, None.

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Organizing Section: LE

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Interspecies analysis revealed that a terminal web-like actin structure is present in fiber ends adjacent to lens sutures of rat, rabbit, pig, and guinea pig, but not chicken. The objective of this ongoing study is two-fold: first, to determine whether a terminal web-like apparatus is present at apical and/or basal ends of human lens fibers, and second, to assess the functional significance of the terminal web-like structure via laser scan analysis in the animal models.

Methods: Human donor lenses (17-40 years old) were fixed, Vibratome-sectioned and labeled with phallolidin-FITC. F-actin was visualized in both anterior and posterior polar sections by confocal microscopy. Functional parameters (focal length and focal variability) were evaluated by laser scan analysis after Cytochalasin-D treatment on rabbit, pig, and guinea pig lenses, all of which possessed the web-like structure. Contralateral lenses were utilized as untreated controls. Statistical comparisons (Wilcoxon rank sum test) were conducted to compare functional parameters in control versus experimental lenses.

Results: In human donor lenses, no evidence of an F-actin web was noted at anterior ends of fully elongated fibers. Posterior fiber ends showed evidence of a web-like F-actin structure which was structurally underdeveloped in relation to "terminal web" seen in rodent lenses. Cytochalasin-D treatment resulted in disruption of the actin web and consequent disorganization of fiber ends in all experimental animal lenses. Laser scan analysis revealed that Cytochalasin-D treated lenses had a greater average focal length and increased focal variability as compared to controls in both rabbits and pig lenses. However, preliminary laser scan data for guinea pigs did not show any significant difference between control and experimental lenses.

Conclusions: Interspecies analysis revealed that a terminal web-like actin structure is present adjacent to posterior sutures in rabbit, pig, guinea pig, and human lenses, but not in chicken lenses. The data indicates that the "terminal web" function to stabilize fiber end organization and its disruption leads to degradation of focal activity. A highly developed F-actin web-like apparatus is likely to restrict fiber end movement, thereby contributing to posterior fiber development and lens maturation.

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2630 - A437

Leaders and Followers: Wound Healing in a PCO Model


Purpose: An essential element of epithelial sheet wound healing is the regulated migration of the epithelial cells into the wound area. We examined the mechanisms that drive and regulate collective cell migration during tissue repair in the lens using a unique ex vivo wound healing model originally developed to study PCO, where cell response to injury is studied in a native microenvironment. These studies focused on the distinct functions of leader and follower cells in wound repair.

Methods: Mock cataract surgery in chick embryo lenses created a highly reproducible wound area. Properties of leader and follower cells in the wound healing model were analyzed by confocal microscopy following immunostaining.

Results: Following mock cataract surgery a distinct subpopulation of vimentin-rich cells that were associated with the anterior aspects of the lens epithelium quickly migrated to the wound edge where they exhibited properties of leader cells that guide the repair of epithelia. These mesenchymal cells lacked cortical Z0-1. Some leader cells had protrusions oriented in the direction of migration while other leader cells were oriented perpendicular to the wound edge with actin filaments organized as a purse string. The protrusions of the leader cells had few actin filaments but were rich in vimentin filaments linked to paxillin-containing focal adhesions. The lens epithelial cells were the follower cells, moving forward as a collective sheet that maintained close Z0-1-rich apical cell-cell junctions but had remodeled their N-cadherin cell-cell junctions. In the dynamically moving follower cells situated just behind the leader population N-cadherin junctions were present in overlapping intermembranous networks while paxillin and actin were found in cryptic lamellipodia polarized toward the wound edge.

Conclusions: The wounded lens epithelium behaves as follower cells with cryptic contactin/actin-rich lamellipodia likely responsible for forward movement of the epithelium. These epithelial cells are led by a subpopulation of vimentin-rich mesenchymal cells whose rapid migration to the wound edge suggests a central role as regulators of the wound-healing process.

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2631 - A438

Topotecan Prevents Induction of Hypoxia-Inducible Factor-1α in Human Lens Epithelial Cells: Implications for Hypoxia-Mediated Regulation of Extracellular Signal-Regulated Kinase (ERK)

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Purpose: Hypoxia-inducible factor-1α (HIF-1α) is expressed in the human lens and may be associated with mitochondrial protection against membrane permeability transition in human lens epithelium (HLE) via activation (i.e. phosphorylation) of ERK. It has been demonstrated in several other cell types that HIF-1α-activated ERK is inactivated through phosphatase removal by mitogen-activated protein kinase phosphatase-1 (MKP-1). The molecular inter-relationship between HIF-1α levels, MKP-1 expression and pERK content is neither well characterized nor understood. We investigated the effect of the anti-cancer drug, Topotecan (Topo), a topoisomerase I linhibitor known to impede hypoxia-mediated HIF-1α induction, with the intent of determining resulting downstream influence on pERK quantity in cultured HLE-B3 cells.

Methods: Protein expression of HIF-1α, MKP-1, ERK and pERK was ascertained by Western blot analysis.

Results: Hypoxia results in the robust induction of HIF-1α protein expression in a time-dependent manner. pERK levels are transient, peaking within 1 hr of hypoxia and diminishing thereafter, despite continuous application of hypoxia. Pretreatment of cells with Topo significantly diminished hypoxia-induced HIF-1α in a dose-dependent manner. Topo pretreatment also resulted in a sustained over-accumulation of hypoxia-activated pERK. Under the same conditions, MKP-1 protein expression was markedly lowered.

Conclusions: MKP-1 is a hypoxia responsive protein, regulated by HIF-1α induction. Hypoxia activated ERK, in human lens epithelial cells, is inactivated through dephosphorylation by MKP-1. Topo is a potent inhibitor of the HIF-1α subunit in hypoxic HLE-B3 cells, leading to decreased MKP-1 expression and over-accumulation of pERK. Taken together, the data is consistent with the fact that hypoxia induces HIF-1α levels resulting in elevated MKP-1 expression; thereby providing a level of homeostatic regulation on the amount of ERK phosphorylation. pERK accumulation is enhanced or reduced by MKP-1 suppression or MKP-1 expression, respectively. pERK has previously been shown to be a critical component in the lens epithelial cell death and protection pathway (Low et al., Am J Physiol, 2008). We are interested in determining whether this artificial enhancement of pERK via intervention by Topotecan, under hypoxic condition, provides enhanced mitoprotection upon reintroduction of oxygen.

CR: J. Pack, None; P.R. Cammarata, None.

Support: None
2634 - A441
Lovastatin Influences TGF-β–Induced Myofibroblast Transdifferentiation in Lens Epithelial Cells
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Purpose: To determine whether lovastatin, HMG-CoA-reductase inhibitors, influences TGF-β–induced myofibroblast transdifferentiation in porcine lens epithelial cells (LECs).
Methods: Porcine LECs were cultured in Dulbecco’s Modified Eagle Medium (DMEM) for 24 hours. Cells were pretreated with or without lovastatin (10μM) for 18 hours and subsequently were stimulated with or without TGF-β(5ng/ml) for 24 hours. To evaluate the expression of α-SMA, α-SMA protein expression was studied by Western blot. Cell contractility was determined in collagen gel contraction assays.
Results: Lovastatin inhibited TGF-β–induced α-SMA expression. In the absence of lovastatin, TGF-β led to a significant increase protein expression after 24 hours of stimulation. Lovastatin strongly diminished the TGF-β–induced increase of α-SMA protein levels. Control gels cultured with DMEM alone contracted to 51.9±4.2% of their original area after 24 hours. TGF-β–significantly increased this contraction (22.3±4.9% of initial gel size; p<0.001, ANOVA). Lovastatin blocked the TGF-β–induced collagen gel contraction (38.6±5.6% of initial gel size; p<0.001, ANOVA).
Conclusions: Lovastatin inhibits TGF-β–induced myofibroblast transdifferentiation of cultured porcine LECs.
CR: C. Urakami, None; S. Kishimoto, None; Y. Tezuka, None; H. Nishigori, None; D. Kurosaka, None.
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2635 - A442
Effect of H-7 on Secondary Cataract After Phacoemulsification in the Live Rabbit Eye
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Purpose: To determine if the serine-threonine kinase inhibitor H-7 inhibits secondary cataract after extracapsular lens extraction by phacoemulsification in the live rabbit eye.
Methods: Eighteen albino rabbits underwent extracapsular lens extraction by phacoemulsification in one eye. The eye was treated with intravitreal H-7 (300 or 1,200μM; n= 6 or 5) or BSS (n=7) immediately after the surgery and twice weekly for 10 weeks. Each eye received slit lamp biomicroscopy once a week, during which the cornea status, intraocular inflammation (anterior chamber flare and cells) and posterior capsule opacification (PCO) were evaluated. The eye was then enucleated and the lens capsule was prepared, fixed and imaged. PCO was evaluated again on the isolated lens capsule under a phase microscope. Soemmerring’s ring area (SRA) and the entire lens capsule area (the area measured along the capsule equator) were measured from capsule images on a computer and the percentage of SRA in the entire capsule area (PSRA) was calculated. Wet weight of the net capsule (WW) was determined on a balance.
Results: No drug-induced corneal edema was observed. Intraocular inflammation in the H-7–treated eye, especially in the 1,200μM H-7–treated eyes, was slightly more apparent than in the BSS–treated eye. No significant difference in PCO was observed in any comparisons. No significant differences in SRA, PSRA and WW were observed between the 300μM H-7–treated eye and the BSS–treated eye. However, differences in SRA (28.3±16.2 mm² vs. 61.4±8.86 mm²), PSRA (33±20% vs. 65±13%) and WW (65.6±279 mg vs. 125±73.7 mg) between the 1,200 μM H-7–treated eye and the BSS–treated eye were statistically significant by the 2-tailed Mann-Whitney U test (P=0.005, P=0.018 and P=0.018 respectively) or by the 2-tailed unpaired t-test (P<0.001, P=0.01 and P=0.01 respectively).
Conclusions: This study indicates that 1,200μM intravitreal H-7 inhibits Soemmerring’s ring formation in the live rabbit eye by ~50%, suggesting that agents that inhibit the actomyosin system in cells may prevent secondary cataract after phacoemulsification.
CR: B. Tian, None; G.A. Heatley, None; M.S. Filla, None; P.L. Kaufman, None.
Support: Grants from NIH (R21 EY017612, R01 EY02698 and P30 EY016665) and Research to Prevent Blindness.
Tuesday, May 4, 1:45 PM - 3:30 PM  Palm A  Symposium  Program Number Range: 2923 - 2930

338. Non-crystallin Functions of Lens Proteins in Health and Disease – Minisymposium  
Organizing Section: LE  
Contributing Section: RE

2923 - 1:45 PM  
Introduction
K.J. Lampi. Integrative Biosciences, Oregon Health and Science University, Portland, OR.

Crystallins have traditionally been thought of as structural proteins until over 10 years ago when alpha-crystallin was determined to have chaperone activity. Emerging data to be presented suggest crystallins may be multifunctional and that defects in their structure lead to disease in ocular tissues and non-ocular tissues.

Support: NIH Grant EY012239

2924 - 1:50 PM  
Involvement of Alpha-crystallin in Ocular Diseases and Other Diseases
J. Horwitz. UCLA School of Medicine, Jules Stein Eye Institute/UCLA, Los Angeles, CA.

Alpha-crystallin a major protein of the mammalian eye lens is known to be involved with congenital cataracts. Mutations in alpha B or alpha A are known to cause recessive or dominant cataracts. Mutations in the beta sheet structure of the conserved “alpha-crystallin domain” involving residues 83 to 120 of alpha A are known to cause autosomal dominant cataract. The mutations in alpha A that are known to date include G98R, R116C, R116H and Y118D. Interestingly, all of the above mutations share common physical-chemical properties. Outside the lens alpha A and alpha B are found, albeit at a much lower levels in the cornea, and retina. In age dependent macular degeneration the levels of alpha crystallin in the retina are significantly higher. The association of increased levels of alpha B crystallin with many neurological diseases such as Alzheimer’s disease, Parkinson’s disease, Alexander’s disease, multiple sclerosis, etc is well documented. More recently alpha-crystallin was shown to be a biomarker for various types of cancer such as breast cancer, head and neck cancer, and non-small cell lung cancer. Alpha-crystallin is recognized to have multiple functions. Its complete 3 dimensional structure and its function outside the lens is yet to be determined.

CR: J. Horwitz. None.
Support: NIH Grant EY 03897

2925 - 2:05 PM  
Repair of α-crystallin Chaperone Activity by MsrA: Implications for α-crystallin Function in the Lens and Other Tissues
M. Kantorow. Biomedical Science, Florida Atlantic University, Boca Raton, FL.

Significant evidence suggests that loss of α-crystallin chaperone function in conjunction with increased methionine oxidation of proteins is a hallmark of lens cataract and other age-related degenerative diseases. We have discovered that a novel repair protein, called MsrA, can repair and restore the chaperone activity of α-crystallin and consistently, the lenses of MsrA knockout mice show increased levels of methionine oxidized and inactivated α-crystallin in association with cataract. Since α-crystallin protection is critical for multiple non-refractive cellular functions ranging from mitochondrial maintenance through apoptosis and protection against oxidative damage, these data suggest that α-crystallin repair is an important modulator of these cellular processes and that loss of α-crystallin repair could play a major role in the development of cataract and other age-related degenerative diseases.

CR: M. Kantorow. None.
Support: EY13022

2926 - 2:20 PM  
αB-crystallin as a Secreted Protein
S.P. Bhat1,2. 1Ophthalmology, Jules Stein Eye Institute UCLA, Los Angeles, CA; 2Brain Research Institute and Molecular Biology Institute, UCLA, Los Angeles, CA 90095, CA.

In a large number of diseases αB-crystallin appears as a component of denatured protein aggregates. The cellular origin of αB-crystallin in these aggregates is not known. We have shown recently that αB-crystallin is secreted from human retinal pigment epithelial cells (ARPE-19) in culture, as exosomes. We have now found that this mode of secretion is not RPE cell - specific but exosomes containing αB-crystallin are also produced from U373 human Glioblastoma cells in culture suggesting that this may be a common mode of αB-crystallin secretion from various cells. We are currently in the process of investigating this mode of secretion using various reporters in various cell types which express αB-crystallin differentially both under normal and stress (heat shock) conditions. Possible implications of αB-crystallin secretion via exosomes will be discussed.

Support: NIH Grant EY00644

2923-2926
We have previously shown that βA3/A1-crystallin is expressed in astrocytes and required for normal remodeling of the developing retina. We now report that βA3/A1-crystallin is also expressed in the retinal pigmented epithelium (RPE). Based on our studies on the Nucl rat, which has a mutation in the βA3/A1-crystallin gene, we believe that this protein is essential in maintaining the homeostasis of RPE cells. Transmission electron microscopy indicates that RPE cells from one month old Nucl rats show abnormal processing of shed photoreceptor discs. Autophagy, the only known process whereby eukaryotic cells degrade and recycle cellular organelles appears to be severely affected in the RPE of the Nucl rat. Immunohistochemistry studies clearly indicate that Microtubule-associated protein 1 light chain 3 (LC3), a marker for autophagy is downregulated in the aging Nucl RPE. Interestingly, the defect does not appear to be in the formation of the autophagosome as our electron microscopic studies show unique perinuclear vacuolar structures containing amorphous and electron-dense material. Moreover, Beclin 1, a protein required for the initiation of the formation of the autophagosome, is expressed in these RPE cells. This suggests that the autophagic clearance of photoreceptor discs in the Nucl RPE is diminished, leading to increased accumulation of cellular wastes in older adult rats. Indeed, as the Nucl rats’ age, RPE cells lose their typical infoldings and the cytoplasm shows increased granularity and lipid inclusions. Based on our findings, we hypothesize that βA3/A1-crystallin is involved in the autophagy process and that disruption of this process, and the resulting inflammatory response, leads to the degenerative disorders that become apparent in the RPE of the aging Nucl rat.

CR: J.S. Zigler, Jr. None.
Support: None.

2929 - 3:05 PM
Mechanism of the Protective Activity of alphaB-crystallin

J.I. Clark. Biological Structure and Ophthalmology, University of Washington, Seattle, WA.

Human alphaB-crystallin is a small stress and heat shock protein (HSP) that is transforming our ideas about endogenous mechanisms for protection against molecular aging and protein unfolding in neurodegeneration, cardiovascular disease, age related macular degeneration and cataract, well known threats to the quality of life in aging humans. While not recognized as protein unfolding diseases, tumorsogenesis, diabetes, and cancer increase in prevalence with age and are associated with the upregulation of alphaB-crystallin, an association which may be consistent with interactions between stress proteins and cytoskeletal assembly or regulatory proteins that maintain normal cellular structure and function. The interactive sequences in the alphaB-crystallin molecule were identified using pin array technology and site directed mutagenesis, and their activities were confirmed using synthetic bioactive peptides based on the interactive sequences. In alphaB-crystallin, the C- and N-terminal extensions protrude from the α-crystallin core domain as unstructured, flexible sequences. In contrast, the core domain is an immunoglobulin-like sandwich of β-strands stabilized by two antiparallel β-sheets. Current findings support the hypothesis that the mechanism for the protective activity of small stress proteins is unique and involves multiple interactive sites for normal self assembly of cytoskeletal proteins as well as abnormal assembly of amyloid fibrils. It is more important than ever before to characterize the biological mechanisms responsible for the actions of stress response proteins, represented by human alphaB-crystallin and the fundamental molecular properties that maximize their protective effects. Supported by grant EY04542 from the NEI.

Support: NIH Grant EY04542

2928 - 2:50 PM
Lessons from Lens Crystallins: The Universality and Implications of Gene Sharing

J. Piatigorsky. Lab Molec Develpmntl Bio/MSC704, National Eye Inst/NIH, Bethesda, MD.

The abundant crystallins are multifunctional proteins responsible for lens refraction. Surprisingly, crystallins are often enzymes or stress proteins that differ among taxonomic groups. Certain ubiquitously expressed proteins also accumulate in the cornea in a taxon-specific fashion, consistent with their having a specialized, although still undefined, optical role. Gene sharing refers to a single protein serving two or more distinct molecular functions, as do crystallins. Gene sharing is not just another name for the recruitment, co-option or hijacking of a protein from one function to another during evolution. Those processes imply loss of the original function with acquisition of the new function. Rather, gene sharing is a universal process whereby a protein has two or more molecular (not just phenotypic) functions depending on its cellular environment and concentration. Gene sharing indicates that a new protein function may evolve by differential expression of its gene and does not require gene duplication or amino acid changes. The use of non-homologous proteins for analogous functions illustrates the importance of convergence in evolution, even within homologous structures (i.e. lens). Gene sharing raises a fascinating, counter-intuitive paradox, namely, that specialization and diversification of proteins go hand-in-hand. Gene sharing has clinical implications concerning disease etiology, functional significance of mutations, gene therapy, and use of animal models for disease. Gene sharing even challenges the definition and boundary conditions of a gene. Thus, gene sharing by lens crystallins has taught us important lessons of general significance for evolution and medicine.

Support: None

2927 - 2:35 PM
BetaA3/A1-crystallin in the Retinal Pigmented Epithelium

J.S. Zigler, Jr. Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD.

We have previously shown that βA3/A1-crystallin is expressed in astrocytes and required for normal remodeling of the developing retina. We now report that βA3/A1-crystallin is also expressed in the retinal pigmented epithelium (RPE). Based on our studies on the Nucl rat, which has a mutation in the βA3/A1-crystallin gene, we believe that this protein is essential in maintaining the homeostasis of RPE cells. Transmission electron microscopy indicates that RPE cells from one month old Nucl rats show abnormal processing of shed photoreceptor discs. Autophagy, the only known process whereby eukaryotic cells degrade and recycle cellular organelles appears to be severely affected in the RPE of the Nucl rat. Immunohistochemistry studies clearly indicate that Microtubule-associated protein 1 light chain 3 (LC3), a marker for autophagy is downregulated in the aging Nucl RPE. Interestingly, the defect does not appear to be in the formation of the autophagosome as our electron microscopic studies show unique perinuclear vacuolar structures containing amorphous and electron-dense material. Moreover, Beclin 1, a protein required for the initiation of the formation of the autophagosome, is expressed in these RPE cells. This suggests that the autophagic clearance of photoreceptor discs in the Nucl RPE is diminished, leading to increased accumulation of cellular wastes in older adult rats. Indeed, as the Nucl rats’ age, RPE cells lose their typical infoldings and the cytoplasm shows increased granularity and lipid inclusions. Based on our findings, we hypothesize that βA3/A1-crystallin is involved in the autophagy process and that disruption of this process, and the resulting inflammatory response, leads to the degenerative disorders that become apparent in the RPE of the aging Nucl rat.

CR: J.S. Zigler, Jr. None.
Support: None.

2929 - 3:05 PM
Mechanism of the Protective Activity of alphaB-crystallin

J.I. Clark. Biological Structure and Ophthalmology, University of Washington, Seattle, WA.

Human alphaB-crystallin is a small stress and heat shock protein (HSP) that is transforming our ideas about endogenous mechanisms for protection against molecular aging and protein unfolding in neurodegeneration, cardiovascular disease, age related macular degeneration and cataract, well known threats to the quality of life in aging humans. While not recognized as protein unfolding diseases, tumorsogenesis, diabetes, and cancer increase in prevalence with age and are associated with the upregulation of alphaB-crystallin, an association which may be consistent with interactions between stress proteins and cytoskeletal assembly or regulatory proteins that maintain normal cellular structure and function. The interactive sequences in the alphaB-crystallin molecule were identified using pin array technology and site directed mutagenesis, and their activities were confirmed using synthetic bioactive peptides based on the interactive sequences. In alphaB-crystallin, the C- and N-terminal extensions protrude from the α-crystallin core domain as unstructured, flexible sequences. In contrast, the core domain is an immunoglobulin-like sandwich of β-strands stabilized by two antiparallel β-sheets. Current findings support the hypothesis that the mechanism for the protective activity of small stress proteins is unique and involves multiple interactive sites for normal self assembly of cytoskeletal proteins as well as abnormal assembly of amyloid fibrils. It is more important than ever before to characterize the biological mechanisms responsible for the actions of stress response proteins, represented by human alphaB-crystallin and the fundamental molecular properties that maximize their protective effects. Supported by grant EY04542 from the NEI.

Support: NIH Grant EY04542
Role of Krüppel-Like Factor 4 in Mouse Lens Gene Expression, Development and Function

D. Gupta, D. Kenchegowda, J. Piatigorsky, S.K. Swamynathan, 1Department of Ophthalmology, Eye and Ear Institute, UPMC, Pittsburgh, PA; 2Laboratory of Molecular and Developmental Biology, National Eye Institute, NIH, Bethesda, MD; 3Department of Ophthalmology, Univ. Pittsburgh School of Medicine, Pittsburgh, PA.

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

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

Results: Expression of Klf4, first detected in the embryonic day-12 (E12) mouse lens, peaked at E16, and steadily declined with age. Mean diameter of the 8 week-old Klf4CN lens was 58% of the age matched WT lens. Klf4CN lens developed structural deformity and central opacity, unlike the normal WT lens. Gene expression comparison by microarray identified 226 and 276 genes up- and down-regulated by more than 2-fold, respectively, in the Klf4CN lens. Alx12 and Alx15 were up-regulated while several crystallins were down-regulated in the Klf4CN lens. Co-transfection with pCI-KLF4 stimulated the Alx12, Alx12cr, Alx15, and Stopbox-crystallin promoter activities by 4- to 20-fold. Pathway analysis identified the lipoxigenase pathway as one of the significantly affected pathways in the Klf4CN lens. Reduced glutathione (GSH) levels were lower while oxidized glutathione (GSSG) levels were higher in the Klf4CN lens than the WT, indicating that the Klf4CN lens is oxidatively stressed.

Conclusions: The expression of Klf4 is developmentally regulated in the mouse lens, where it contributes to different pathways including arachidonic acid metabolism, aryl hydrocarbon receptor signaling, and glutathione metabolism. CR: A. Cvekl, None; C. Yang, None; Y. Yang, None; L.A. Brennan, None; E. Bouchassira, None; M. Kantorow, None. Support: NIH R01 EY012200, EY014237 and EY013022.

The Circulation of Sodium and Fluid Through Lens Gap Junctions

R.T. Mathias, J. Gao, X. Sun, L. Moore, T.W. White, Physiology & Biophysics, State Univ of NY-Stony Brook, Stony Brook, NY.

Purpose: A fundamental question on the biophysical properties of gap junction channels is whether their role in transporting fluid through stratified epithelia is limited to the apical-lateral directions, as is the case in other tissues, or whether their role in transporting fluid through the entire lens is conserved as well. Surprisingly, no previous data address this question. We recently modeled fluid flow through gap junction channels of the ciliary body, and concluded the channels could indeed carry near isotropic fluid, but flow would be driven by hydrostatic pressure rather than osmotic pressure. The pressure required to drive fluid through a single layer of gap junctions might be small and difficult to measure, but in the lens, intracellular fluid flow from central fibers to the surface crosses hundreds of layers of gap junctions and should require a large hydrostatic gradient.

Methods: A high-speed pressure sensing system was constructed to measure intracellular hydrostatic pressure in the lens.

Results: In wild type (WT) mouse lenses, the measured pressure gradient varied from an average of 330 mmHg at the center to zero at the surface. In Cx46 for Cx50 knockouts (KO) lenses, mature fiber cell coupling conductance is 2-2.2 times higher than that of WT. Hydrostatic pressure in KO lenses varied from an average of 150 mmHg at the center to zero at the surface, or about 2.2-2 times lower than that in WT. In GPX knockout (KO) lenses, mature fiber cell gap junction coupling is 1.7-times lower than that of WT. Hydrostatic pressure in KO lenses varied from an average of 490 mmHg at the center to 0 at the surface, or about 1.5-times higher than that in WT. When WT lenses were bathed in high potassium low sodium solution to block the normal circulation of sodium, the hydrostatic pressure went to zero over the same time period as the circulation of sodium went to zero.

Conclusions: These data suggest that is indeed a vigorous circulation of fluid through the lens, the intracellular layer of fluid circulation is through fiber cell gap junction channels, and the fluid flow is coupled to the lens circulation of sodium. CR: R.T. Mathias, None; J. Gao, None; X. Sun, None; L. Moore, None; T.W. White, None; P.R. Brink, None. Support: NIH grant EY06391.

High-Efficient Conversion of Human Embryonic Stem Cells Into Differentiated Lens Progenitor Cells and Lentoid Bodies

A. Cvekl, C. Yang, Y. Yang, L.A. Brennan, E. Bouchassira, M. Kantorow. Ophthalmic & Vis Sci & Genetics, Medicine & Cell Biology, Albert Einstein Coll of Medicine, Bronx, NY; Biomedical Sciences, Florida Atlantic University, Boca Raton, FL.

Purpose: This study sought to establish procedures to derive properly differentiated lens cells from undifferentiated human embryonic stem (hES) cells.

Methods: To generate large quantities of lens progenitor cells and differentiated lens cells, a human embryonic stem (hES) cell line called H1 was exposed to growth factors known to regulate lens development and the optimal sequence, timing and concentration of these factors was determined. Differentiation of hES cells was monitored by examining the sequential expression of lens crystallins and other markers of lens differentiation between the differentiated lens cells and actual human lens samples by quantitative RT-PCR, immunofluorescence and western immunoblotting. The morphology of the differentiated lens cells was also evaluated by electron microscopy.

Results: Using growth factors known to regulate ectoderm and lens placode formation (BMP4, BMP2, FGF2 and Wnt5a), in a two-stage differentiation procedure, a majority of cells cultured for 12 days expressed Pax6, a lens-lineage specific transcription factor. After 15 days in culture, these cells subsequently expressed both of αA and αB crystallins. Next, various combinations of FGF2/Wnt3a/BMP4 and BMP7 were tested to examine lentoid body formation indicative of achieving the three-dimensional biconcave structures required for mature lens function. The most efficient production of these structures, at day 35 of culture, was found using FGF2 and Wnt5a.

Conclusions: These studies identify a novel procedure to generate differentiated human lens cells from human embryonic stem cells specific induced pluripotent (iPS) cells. These de novo differentiated lens cells provide a unique tool to study diverse biological processes including progenitor lens formation, epithelial cell differentiation, fiber cell differentiation, lens protection and cataractogenesis.

CR: A. Cvekl, None; C. Yang, None; Y. Yang, None; L.A. Brennan, None; E. Bouchassira, None; M. Kantorow, None. Support: NIH R01 EY012200, EY014237 and EY013022.

Activation of the Unfolded Protein Response During Normal Lens Development

Z. Firtina, M.K. Duncan. Biological Sciences, University of Delaware, Newark, DE.

Purpose: The Unfolded Protein Response (UPR) is a cellular stress pathway activated in response to accumulation of unfolded proteins in the Endoplasmic reticulum (ER). We have shown that UPR is highly activated by retention of extracellular matrix proteins in the lens resulting in cataract. However, UPR activation is also required for professional secretory cell development to drive the necessary ER expansion. Since lens fiber cell differentiation requires the synthesis of many new membrane proteins, we tested the hypothesis that UPR is activated during lens fiber cell differentiation.

Methods: Lenses were extracted from embryonic and newborn FVB/N mice. The molecular markers of UPR were evaluated by immunohistochemistry, RT-PCR and western blotting.

Results: Normal lenses show activation of all three UPR pathways during development. IRE1 is an ER-localized transmembrane protein that, when activated, performs a site-specific cleavage of Xbp1 mRNA to produce a transcription factor. In normal lenses we detected the splicing of Xbp1 mRNA and the production of the XBP1(S) protein in E15.5 and E16.5 lenses. The second UPR pathway is mediated by ATF6, a transmembrane protein that, upon ER stress, undergoes proteolytic cleavage enabling its cytosolic domain to translocate into the nucleus as a transcription factor. In normal lenses, ATF6 was first detected in the nucleus of lens cells undergoing primary fiber cell elongation at E11.5. The third arm of the UPR is mediated by PERK which, upon activation, phosphorylates eIF2α causing a general attenuation in protein translation. The active form of PERK was detected at the apical tips of lens fiber cells at E12.5 and E13.5 and E16.5 embryonic lenses. Phosphorylation of eIF2α also induces selective translation of some mRNAs such as ATF4 which is a transcription factor regulating many stress related genes. ATF4 expression was first detected in the nucleus of E11.5 lens cells undergoing primary fiber cell elongation.

Conclusions: Overall, these data show that UPR is activated during normal lens development and support the hypothesis that UPR may be important in the development and differentiation of the normal lens.

CR: Z. Firtina, None; M.K. Duncan, None. Support: EY015279.
Activation of the Unfolded Protein Response (UPR) in Connexin50 Mutant Lenses

M.K. Duncan, J. Stull, Z. Firtina. Biological Sciences, University of Delaware, Newark, DE.

Purpose: We have previously found that retention of newly synthesized collagen IV in the endoplasmic reticulum (ER) leads to high level activation of UPR, a cell stress response pathway, resulting in cataracts. Further, it has been reported that mice harboring mutations in connexin50 (Cx50) have more severe lens phenotypes than those lacking the gene. Here we test the hypothesis that retention of mutant Cx50 within the ER leads to robust UPR and can contribute to the increased severity of cataracts in Cx50 mutant mice.

Methods: Embryonic, newborn and adult eyes were obtained from mice homozygous for the Cx50S50P and Cx50G22R alleles. Lens morphology was assessed by dark-field microscopy and histological analysis. The expression of UPR markers in comparison to wildtype controls was assessed by western blotting, rt-PCR and immunolocalization in lent obtained from animals ranging from embryonic day 12.5 until adulthood.

Results: As previously reported, both Cx50S50P and Cx50G22R mutant lenses are small. Have an unorganized fiber cell structure, and cataract. We found that lenses from both of these genotypes begin to upregulate the expression of the ER resident chaperone Bip compared to wildtype lenses in mid-embryogenesis and this relative upregulation is robust by birth. While the full gamut of pathways responsible for this upregulation are still under investigation, the Irel pathway appears to be inappropriately activated as measured by the elevated levels of Xbp-1 splicing detected in mutant relative to wildtype lenses.

Conclusions: Expression of mutant transmembrane proteins in the lens can lead to the activation of UPR. This supports the hypothesis that UPR can contribute to the lens phenotypes of Cx50 mutants.

CR: M.K. Duncan, None; J. Stull, None; Z. Firtina, None.

Support: NIH Grant EY015279, Fight for Sight Basil V. Worgul Fellowship in Lens Research, Howard Hughes Medical Institute.

Spatial Distributions of Aquaporin 0 and MP20 Modifications in Human Lenses


Purpose: To determine the location and extent of modification to lens membrane proteins, AQ0P and MP20, in human lenses with age.

Methods: AQ0P and MP20 tryptic peptides were generated from digestion of lens membrane protein preparations from manually dissected lens regions of young (18-23y) and older (51-60y) lenses. Quantitative proteomics methods (LC-MS/MS) with isotope labeled internal standards (AQUA peptides) were used for absolute quantitation of AQ0P and MP20 phosphorylation levels in each lens region. In addition, MALDI tissue profiling and imaging were carried out on frozen lens sections after water washes to determine the extent of AQ0P truncation and fatty acid modification to AQ0P. On tissue Lys-C digestion allowed distinction between N- and C-terminal fatty acid modification.

Results: A peak in phosphorylation for both AQ0P and MP20 was observed in the outer cortical region (average r/a = 0.83-0.90) in all lenses. At an r/a of approximately 0.76 the level of AQ0P 5235 phosphorylation decreased in older lenses and was significantly different than the levels in younger lenses. A similar pattern was observed for MP20 SI70 phosphorylation. MALDI imaging of AQ0P revealed extensive truncation starting at the inner cortical region in older human lenses. MALDI profiling of on tissue digested AQ0P revealed little fatty acid modification in the outer cortex, but up to 50% modification in the nuclear region (r/a = 0.35-0.6). Moreover, the results indicated that a majority of the fatty acid modification is positioned at the N-terminus.

Conclusions: The significant decrease in AQ0P phosphorylation in older lenses occurs in the region identified as a “barrier” region. Thus, the regulation of AQ0P permeability by AQ0P phosphorylation, which affects calmodulin binding, may play a role in establishing a permeability barrier in older lenses. The functional consequence of fatty acid modification has yet to be determined; however, the major N-terminal site could be involved in protein trafficking or anchoring.

CR: K.L. Schey, None; D.B. Gutierrez, None; Z. Wang, None; A.C. Grey, None; D. Garland, None.

Support: EY13462

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CR: K.L. Schey, None; D.B. Gutierrez, None; Z. Wang, None; A.C. Grey, None; D. Garland, None.

Support: EY13462
Sprouty Prevents TGFβ-Induced Cataractogenesis

E.H. Shin1, M.L. Robinson1, M.A. Basson1, G. Martin1, F.J. Lovicu1, K.R. Hegde1
1Anatomy and Histology, Bosch Institute & Save Sight Institute, University of Sydney. The Vision Cooperative Research Centre, University of NSW, Australia; 2Department of Zoology, Miami University, Oxford, OH; 3Department of Craniofacial Development, King's College London, London, United Kingdom; 4Department of Anatomy, University of California, San Francisco, CA.

Purpose: Cataract, the loss of transparency of the lens, is one of the leading causes of blindness in the world. A common form is anterior subcapsular cataract (ASC), characterized by an epithelial-to-mesenchymal transition (EMT). Maintenance of the lens is achieved by growth factors and their respective receptor-mediated signaling pathways. Members of the Sprouty (Spry) family are negative regulators of such pathways. In our laboratory, Spry1 and Spry2 have previously been shown to be expressed in the lens, primarily in the lens epithelium. More recently, we have demonstrated that conditional deletion of Spry1, or Spry1&2 results in ASC formation.

The purpose of our current study was to determine the molecular mechanisms in the absence of Spry leading to ASC formation. Furthermore, since ASC is a characteristic phenotype observed in TGFβ-overexpressing lenses, we hypothesize that Spry1 overexpression will prevent or reduce ASC formation.

Methods: Mice conditionally deficient for Spry1 or Spry1&2 in the eye, or lens, were generated using the established LeCre or ML1Cre transgenic Cre-lines, respectively. For the ASC rescue study, transgenic mice overexpressing Spry1 in the lens were crossed with Spry11/1 LEC mice carrying a TGFβ1 overexpressing transgene (TGFβ1 WT). The lens was excised and the protective effect in vivo using galactosemic animals was evident by its ability to prevent ASC formation. In vitro studies were conducted in rat lens fiber layers of the ARCs but not in the clear lenses. Since, ER stress is induced only in LECs, ROS must come from LECs. We will further discuss potential mechanisms of an activation of the UPR and the production of ROS.

Results: The deletion of Spry1 or Spry1&2 in the lens led to vacuolization of the epithelial ASC formation. However, the posterior lens capsule thinned and subsequently ruptured. Prior to ASC formation, there was aberrant (increased) activation of TGFβ signaling (increased labeling of pAkt and Snail) in lens epithelia of Spry-deficient lenses compared to WT lenses. When Spry1 was overexpressed in TGFβ1-overexpressing lenses, there was no evidence of ASC formation. The development of ASC in Spry-deficient lenses suggests that Spry may play a protective role for the normal lens epithelium. This is supported by the ability of Spry1 overexpression in TGFβ1-inhibiting lenses. Overall, Spry proteins in the lens may be important for negatively regulating the signaling pathways (including TGFβ signaling) required for maintenance of the normal lens epithelium, and may serve as a putative therapeutic agent for the prevention of ASC in situ.

Conclusions: The UPR activation might play a significant role in human ARCs. Our results further suggest that peripheral LECs under ER stress produce ROS to further oxidize the lens.

Support: NIH Grant EY0-3177, NHMRC

Antioxidant Effects of Caffeine. Prevention of Lens Damage and Cataract Formation

S.D. Varma1,2, K.R. Hegde3, S. Kortun1

Purpose: Intracellular generation of reactive species of oxygen in the lens and aqueous and consequent physiological damage to the tissue has been shown to be involved in the genesis of cataracts in experimental animals as well as in humans. The present study was designed to examine the feasibility of inhibiting this process by caffeine, an alkoaloid present in many common beverages.

Methods: Oxidative effects were studied in vitro as well as in vivo. In vitro studies were conducted by incubating mice lenses in medium exposed to UV (365nm) in the presence of caffeine with and without caffeine (Spry1). The extent of lens damage was assessed by determining the ability of the tissue to conduct active transport of 86Br and by measuring the levels of GSH and ATP. In vivo studies were conducted in rats by incorporating caffeine with galactose in the diet and determining the levels of GSH, ATP and hydration index of the lenses, combined with morphological studies.

Results: Incubation of the lenses under UV in the presence of kynurenine lead to substantial inhibition of active cation transport as well as decreases in the levels of GSH and ATP. These deleterious effects were significantly prevented by caffeine. Its protective effects on normal lens animals was evident by its ability to prevent the early loss of GSH, the first index of oxidative damage in this animal model. Lens hydration and ATP were also better maintained. The anti-cataractogenic effects were also strongly apparent ophthalmoscopically, ascertained further morphologically. These results in vitreous damage by oxidative radicals generated photochemically, as well as in vivo by its ability to modulate the cataractogenic process in galactose-fed animals. These effects of caffeine have not been reported before and are hence considered highly interesting in the view of its relatively high content in widely consumed beverages. ESR findings combined with its ability to prevent GSH depletion suggest that these effects are attributable to its property of scavenging ROS. However, additional modes of its action and their pharmacological significance are under investigation.

Support: NIH Grant EY01192

Activation of the Unfolded Protein Response (UPR) in Age-Related Cataract Lenses

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Purpose: Age-related cataracts (ARCs) are a multi-factorial disease with a poorly understood etiology. Oxidative stress, combined with aging of the lens is believed to contribute to ARC formation, but sources of the reactive oxygen species (ROS) remain elusive. The unfolded protein response (UPR) activates the production of ROS in various tissues. So far there is no information whether human ARCs are associated with activation of the UPR and ROS productions in LECs. Here we investigated the presence of UPR-specific proteins in the lenses of ARCs.

Methods: Pairs of clear human lenses from different age groups and various types of ARCs were obtained from the National Disease Interchange (NDRI), Philadelphia, PA. One lens from each pair was used for protein analysis and the other lens was used for immunohistochemical analysis. Massspectroscopic analysis was used to authenticate UPR-specific proteins after purification with antibody precipitation and SDS-PAGE fluctuation.

Results: Protein blot analysis showed that clear lenses at ages 17, 30, 44, 63, and 74 did not have apparently detectable levels of UPR-specific proteins. In contrast, in ARCs at similar age groups, significantly higher levels of UPR-specific proteins were found including; ATF4, ATRF, PERK, IRE1 α, Bip, CHOP, Calnexin, procaspase-12, eIF2α, and Dnmts. E3 ubiquitin ligases enzymes for the production of ROS in the UPR such as FasD, E3L, and Erol-L4 were also significantly elevated in ARCs. Immunohistochemistry of the lens thin sections showed multiple immune-positive staining bands in the outer cortex lens fiber layers of the ARCs but not in the clear lenses. Since, ER stress is induced only in LECs, ROS must come from LECs. We will further discuss potential mechanisms of an activation of the UPR and the production of ROS.

Conclusions: The UPR activation might play a significant role in human ARCs. Our results further suggest that peripheral LECs under ER stress produce ROS to further oxidize the lens.

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Mechanism, Specificity, and Significance of Aldose Reductase Inhibition by Curcumin

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Purpose: Accumulation of intracellular sorbitol due to increased aldose reductase (ALR) or A KRIBI activity has been implicated in the development of diabetic eye complications such as cataract formation. Here, we investigated the effects of curcumin, a dietary antioxidant present in the commonly used spice Curcuma longa on aldose reductase activity and on hyperglycemia-induced changes in lens and retina.

Methods: Inhibition of members of aldo-keto reductase (AKR) superfamily, human recombinant aldose reductase (AKRIB1) and human small intestine reductase (AKRIB10) and bovine kidney aldehyde reductase (ALR) by curcumin was determined by spectrophotometric assays. Kinetic constants, Km and Vmax of recombinant AKRIB1, ALR and ALRIB10 were determined with varying concentrations of glyceraldehyde as substrate in presence of curcumin by Lineweaver-Burk double reciprocal plots. Binding constant (Kd) and sorbinil displacement were determined by fluorescence titration with curcumin and sorbinil respectively. Inhibition of AKRIB1 and AKRIB10 and specificity for AKR members for keto and enol forms of three major curcuminoids were determined by molecular docking studies. The potential of curcumin to suppress the formation of sorbitol in red blood cells under high glucose conditions and to prevent by hyperglycemia-mediated changes in lens and retina of diabetic rat was investigated. VEGF expression was measured by western blotting and real time PCR.

Results: Curcumin inhibited aldose reductase (AKRIB1) with an IC50 of 10 μM in a non-competitive manner, but was a poor inhibitor of AKRIB10 and ALR. Molecular docking data were consistent with the pattern of inhibition of AKRIB1 by curcumin which specifically and indicate that both keto and enol forms have similar effects. Moreover, curcumin was able to suppress sorbitol accumulation in red blood cells under high glucose conditions. Further, these results could be translated to in vivo conditions as curcumin was effective in delaying experimentally-induced diabetic cataracts in rats and inhibition of vascular endothelial growth factor (VEGF) in diabetic rat retina.

Conclusions: These results suggest that curcumin might be useful for the treatment and/ or prevention of diabetic ocular complications.

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3820 - 9:30AM
Ubiquitin-Dependent Regulation of Stress Response in Human Lens Epithelial Cells
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Purpose: Up-regulation of heat shock proteins is an important cellular protective mechanism for withstanding and recovering from environmental insults. Celastrol, a tripterpenoid compound isolated from Celastraceae, is a potent inducer of heat shock responses. The objective of this work is to investigate the mechanism by which celastrol induces heat shock response in human lens epithelial cells (HLEC).

Methods: HLEC (SRA 01/04) were treated with 1 μM and 3 μM celastrol. The mRNA levels of cytoplasmic chaperones were determined by Real-Time PCR. Protein levels for these chaperones and ubiquitin conjugates were determined by Western blotting. Proteasome activity was determined using fluorogenic peptides as substrates. Cytoprotective effects of celastrol were determined by MTS assay upon exposure to H₂O₂ or L-canavanine.

Results: Treatment of HLEC with celastrol induced expression of a broad spectrum of cytoplasmic molecular chaperones. Specifically, mRNA levels of αB-crystallin, Hsp27 and Hsp70 increased 20-120-fold upon celastrol treatment. Protein levels of Hsp27, Hsp70 and 90 increased ~ 2-6 fold. Levels of ubiquitin conjugates increased in a dose-dependent manner upon celastrol treatment. Activities of all of the three peptidases of the proteasome decreased 30-50% upon celastrol treatment. Impairment of functions of the ubiquitin-proteasome pathway (UPP) resulted in changes that are similar to those induced by celastrol, indicating that celastrol may induce stress response via inhibition the proteasome. Consistent with the results obtained upon UPP inhibition, celastrol treatment enhanced the toxicity induced by H₂O₂ and L-canavanine.

Conclusions: We have demonstrated that celastrol induces stress response via impairing the proteasome and accumulation of ubiquitin conjugates. Since increasing ubiquitin conjugates by other methods, such as overexpression of ubiquitin or CHIP (a ubiquitin ligase) also induces stress response in HLEC, these data suggest that accumulation of ubiquitin conjugates in cells is a mediator of stress response. The data also indicate that UPP is essential for cells to cope with environmental stress, such as oxidative stress. Thus, age- or stress related impairment of the UPP in the lens may be a contributing factor for the accumulation of damaged proteins and cataractogenesis.

CR: Q. Bian, None; A. Taylor, None; F. Shang, None.
Support: NIH grant EY01717 (to FS); USDA cris 1950-51000-60-1A (to AT)

3822 - 10:00AM
AktIAccelerates Oxidative Apoptosis in HLE-PC Cells
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Purpose: Akt signaling pathway plays a very important role in mediating lens differentiation, development and stress-induced pathogenesis. Oxidative stress has been shown to play a role in cataractogenesis and one of the mechanisms for oxidative stress to induce cataractogenesis occur through induction of lens epithelial cell apoptosis. Thus, one of the mechanisms for Akt to promote survival is to prevent stress-induced apoptosis. In the present study, we have examined the role of Akt1 in regulating oxidative stress-induced apoptosis.

Methods: Stable system to generate hydrogen peroxide was utilized for the oxidative insult on various types of lens epithelial cells. Both vector and AktI transfected human lens epithelial cells were established for this study. Cell flow cytometry and MTT assays were used for detection of apoptosis. Western blot analysis was used to examine the activation of AktI and determine the expression patterns of the apoptosis-related genes including p53 and members of the Bcl-2 family.

Results: Hydrogen peroxide induces apoptosis in both vector- and AktI-transfected cells. However, the apoptosis rate in AktI-transfected stable line is higher than that in vector-transfected cells. Associated with this differentially apoptotic process, we observed that the tumor suppressor, p53 is phosphorylated at Ser-15 and Ser-37 much strongly in AktI-transfected cells than in vector-transfected cells. The p53 target genes, both Bak and Bax are up-regulated more in AktI-transfected cells than in vector-transfected cells.

Conclusions: AktI overexpression accelerates oxidative stress-induced apoptosis in HLE-PC cells.

CR: L. Zhang, None; X. Zeng, None; S. Sun, None; L. Xiao, None; L. Gong, None; M. Deng, None; J. Liu, None; H. Ma, None; D.W. Li, None.
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4565 - D635
An Investigation of New, Real-Time, Intraoperative Aberrometry to Evaluate and Improve Accuracy in IOL Power Selection in Eyes Undergoing Cataract Surgery Following Previous Refractive Surgery


Purpose: To evaluate the accuracy of real-time intraoperative wavefront aberrometry in IOL power selection in eyes undergoing cataract surgery following previous refractive surgery.

Methods: Eyes must have undergone previous uncomplicated LASIK or PRK, have a well-centered topographic ablation profile, and require cataract surgery due to age-related cataracts. A retrospective chart review was performed in which charts were reviewed of twenty patients who underwent phacoemulsification with IOL implantation with an intraoperative wavefront measurement to confirm if the visual target was achieved. Preoperative parameters measured were BCVA, UCVA, cornea curvature, axial length, and manifest refraction. Postoperative parameters include manifest refraction, BCVA and UCVA.

Results: In fifteen eyes, the intraoperative measurement confirmed that the IOL power was accurate, while in five eyes, the measurement indicated the need to exchange the IOL.

Conclusion: Intraoperative wavefront aberrometry enables real-time, intraoperative refractive measurements and improves refractive outcomes after cataract surgery with a previous history of corneal refractive surgery.

CR: R. Solomon, None; E. Dunnenfeld, Wavecet, C.
Support: None

4566 - D636
Phacoemulsification With Multifocal Intraocular Lens Implantation for Bilateral Cataract With Anterior Lenticous

R. Tano, Y. Yamamoto, T. Sakurai, T. Mano. Tane Memorial Eye Hospital, Osaka, Japan.

Purpose: Anterior lenticous (AL) is usually observed in a patient with Alport's syndrome, a hereditary defect in the synthesis of collagen in the basement membrane. In this case report, we perform phacoemulsification with multifocal intraocular lens (IOL) implantation for bilateral cataract with AL. Histological findings of lenticous specimens obtained during surgery was also discussed.

Methods: A 59-year-old man consulted our hospital for applying for refractive surgery. The decimal uncorrected visual acuity (VA) was 0.1 and the best corrected VA was 1.0 in both eyes. The refractive status was –17.50D –1.75D x 70 in the right eye and –18.50D –1.50D x 70 in the left eye. Both lenses showed cataract with AL. No other ocular abnormalities were found. The patient had no signs of hearing loss. Serologic or urine examinations showed normal. Phacoemulsification with multifocal IOL implantation using mix & match approach was performed in each eye (OD: Tecnis® ZM600, AMO; OS: ReZoom™ NXG1, AMO) Anterior capsule was obtained during surgery for histological assessment in transmission electron microscopy (TEM).

Results: Although capsulorhexis tear was made in the right eye during surgery because of the fragility of the anterior capsule, IOL was implanted in the capsular bag as planned. There was no surgical complication in the left eye. The uncorrected VA at 5 m, 1 m and 30 cm at post-op 6 months was 1.2, 0.5, 1.0 in the right eye and 0.9, 0.8, 0.2 in the left eye, respectively, with high patient satisfaction. No remarkable decentration of IOL was observed in both eyes. TEM of the anterior lens capsule showed number of vertical dehiscences in the capsule and numerous lacunae in the cytoplasm.

Conclusions: Successful results were gained in post-operative visual acuity and patient satisfaction by implanting multifocal IOL for bilateral cataract with AL. Capsulorhexis tear should be performed carefully in AL because of the fragility of the capsule.

CR: R. Tano, None; Y. Yamamoto, None; T. Sakurai, None; T. Mano, None.
Support: None

4567 - D637
Simulation of Decentration of Intraocular Lenses With Positive and Negative Spherical Aberration: Effects on Wavefront Error and Optical Quality

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Purpose: For compensation of corneal spherical aberration (SA), intraocular lenses (IOLs) offer SA with negative SA have been developed. Decentering of IOLs can influence refractive error and retinal image quality. This study investigates the effect of micro-decentration of the IOL optic of spherical and aspheric IOL on total wavefront aberration and optical quality in a computer model.

Methods: For 42 eyes of 21 patients corneal wavefront error was calculated by Zernike expansion of axial keratometric topography data over a diameter of 6 mm. Using a computer model, the resulting change in total wavefront aberration from decenteration of a conventional spherical IOL and an aspheric IOL with negative SA in 100 µm steps up to 0.5 mm over a pupil diameter of 5 mm was calculated. The visual Strehl ratio based on the optical transfer function with simulated best-spherical-cylindrical correction (BCVSOTF) was used as a metric for optical quality. A decrease of BCVSOTF by 2 log units was assumed as a criterion for significant deterioration of optical quality.

Results: For the spherical IOL, mean maximum decentration without deterioration of optical quality was 44±14 µm, for the aspheric IOL, 44±25 µm (p=0.78). With decenteration in the direction of the axis of corneal horizontal coma, the aspheric IOL showed a tendency towards an increase of BCVSOTF, the spherical IOL showed the same with decentration in the opposite direction.

Conclusions: Both IOLs showed the same tolerance to decentration for a pupil diameter of 5 mm in the computer model. Slight decentration in the direction of corneal horizontal coma showed a tendency to improve optical quality.

CR: M. Baumeister, None; J. Buhrer, None; T. Kohnen, None.
Support: None

4568 - D638
Patient Satisfaction After Toric Intraocular Lens Implantation

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Purpose: To evaluate patient satisfaction after TORIC IOL, to compare the frequency of spectacle wear (Asthigmatic correction) pre and post-operatively and to assess quality of vision post-operatively.

Methods: A questionnaire based study involving 4 questions: 1-Frequency of spectacle wear post-operatively (always, often or never) 2-Patient satisfaction without glasses (scale 1-10) 3-Frequency of glare (scale 0-4) 4-Asking if patients would select the same lens again (yes or no)

It was carried out via telephone interviews (10-15 minutes) with a total of 46 patients enrolled. All patients had uncomplicated; SN60T3 TORIC IOL implantation in both eyes (2008-2009). We studied the effect of correcting astigmatism on patient satisfaction. Results: 38 patients were successfully reached and agreed to participate. 25 (65%) patients were males and 13 (35%) were females. Their age ranged from 51-83 years and the mean is 67 years. 36 (95%) patients were spectacles before the surgery and 7 (18%) patients only wear spectacles after the surgery. 4 (10.5%) of them wear the spectacles “all the time”, 3 (7.8%) patients “sometimes”. 26 (68.4%) patients report a satisfaction score (10/10 on the scale satisfaction) with 7 (18.4%) patients report 9/10, 3 (7.9%) patients report 8/10 and only 2 (5.3%) patients report less than 6. 35 (92.1%) patients report no glare (0/4 on the glare scale), 2 (5.3%) patients report 2/4 glare and 1 (2.6%) patient has 3/4 glare. 36 patients would choose the same lens again while 2 would not. For those patients who wear glasses post-operatively or gave a satisfaction score less than 6/10 or 3/4 on the glare scale or who would not choose the same lens again have spherical refractive error (-1.5 — -1.25D) and astigmatic error ranging from -0.25—0.75D.

Conclusions: TORIC IOL implantation resulted in high spectacle independence post-operatively and those who needed glasses were due to spherical ametropia and not due to astigmatism. The unfavorable postoperative symptoms (e.g Glare and night vision difficulties) were no different from those published after monofocal intra ocular lenses implantation. We expect greater patient satisfaction with higher Cylinder correction (e.g T4, T5).

CR: M.S. Alkharashi, None; F.M. Alfaradhan, None; M. Discipola, None.
Support: None
Accelerated Deterioration Test of a Foldable Acrylic Intraocular Lens by an Experimental Technique, and the Significance of This Test


**Purpose:** For faster prediction of deterioration of intraocular lenses (IOL), we performed an accelerated deterioration test by using the Arrhenius equation, which is an empirical equation that expresses the principle that “the lower the temperature, the slower a given chemical reaction will proceed, and, conversely, the higher the temperature, the faster a reaction will proceed.” According to the Arrhenius equation, the recommended heating temperatures were 50-60°C, and we calculated the period of the 5-year accelerated deterioration test to be 371 days. We performed a 60°C accelerated-deterioration test on each type of soft acryl IOL, and we investigated whether we could predict the same changes in these tested lenses as in IOLs that have been inserted into a human eye. We predicted the metamorphosis and deterioration of soft acryl IOLs and evaluated these changes after 5 years.

**Methods:** We selected 3 pieces each of 11 kinds of acrylic soft lenses (IOLs). Clear hydrophobic acrylic: MA60BM, SA60AT, AR40e, VA-60BB, N-188, AL6. Colored hydrophobic acrylic: SN60AT, YA-60BB, N-41YB, AN6. Clear hydrophilic acrylic: HP60M. We placed each of the IOLs into a 50-ml screw container containing ultrapure water and let them soak for 371 days in a 60°C oven. After observing the appearance of the IOLs, we measured the changes in their weights, optic diameters, and center thicknesses, and we calculated the rate of these changes before and after the test.

**Results:** Observed deterioration: IOLs that uniformly changed to a glistening-like appearance: MA60BM, SA60AT, YA-60BB, N-188, SN60AT, YA-60BB, N-41YB. IOLs that showed slightly granular glistening: AR40e IOLs that showed glistening only in their centers: AL6, AN6. IOLs that did not show any glistening: HP60M. Rate of weight change: Tended to increase for all of the IOLs. In particular, SA60AT increased 3.5% and AN6 increased 3%. Rate of change of the optic diameter: Increased about 0.4% in AL6 and AN6. Rate of change of center thickness: Decreased about 1.5% in HP60M. Less than ±0.5% in the other IOLs.

**Conclusions:** Some acrylic lenses have molecular structures that retain their soft shape. Based on this accelerated test, we concluded that the opacity of these lenses was caused by the entrance of water molecules into these molecular structures. However, IOLs with water content of 0.5% or more tended to have minimal opacity.

Support: None

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Role of Participation in Clinical Trials on Affecting Patients’ Satisfaction After Receiving Premium IOLs

M. A. Guenena, H. P. Sandoval, K. D. Solomon. Ophthalmology, Magill Research Center, Storn Eye Instit, Charleston, SC.

**Purpose:** Evaluate the role of clinical trial participation in patient satisfaction after multifocal IOL implantation (MFIOL).

**Methods:** 112 patients that received bilateral MFIOLs were divided into two groups. First group (n=56) included patients who participated in clinical trials. Second group (n=56) included those who didn’t. Overall OS, distance-DS, intermediate-IS, near-NS and night vision satisfaction-NTVS was assessed using a scale from 0 (=very dissatisfied) to 10 (highly satisfied).

**Results:** No significant difference in age, residual error, postoperative visual acuity (uncorrected or best-corrected) at distance, intermediate and near between groups. NS was significantly higher in clinical trial patients-CTP (8.5 vs 7.9. P<0.05). Although CTP showed overall higher satisfaction scores; no significant differences was found in other parameters.

**Conclusions:** Trend shows that CTPs tend to be more satisfied than non CTPs. This factor must be taken in consideration when reflecting the clinical trial results onto the general population.

Support: NIHR/NEI EY-014793

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Anti TGF-β2 and MMP Inhibitor Modification of PDMS as a Model IOL to Decrease the Incidence of PCO

B. Amoozgar, H. Sheardown. Biomedical Engineering, McMaster University, Hamilton, ON, Canada.

**Purpose:** Lens epithelial cells (LEC) remaining in the capsular bag following a cataract surgery, influenced by wound healing process, undergo epithelial to mesenchymal transition (EMT) and migrate from the anterior to posterior capsule resulting in the generation of fibroblastic cells, production of alpha smooth muscle actin (α-SMA), deposition of extracellular matrix (ECM), capsule wrinkling, and eventually posterior capsule opacification (PCO). The wound healing process is regulated by growth factors and cytokines especially transforming growth factor beta (TGF-β) and matrix metalloproteinases (MMPs). Delivery of TGF-β inhibitor (K16) or MMP inhibitors including sulfadiazine and tissue inhibitor of metalloproteinase (TIMP-3) via an intraocular lens (IOL) could prevent the cellular changes which lead to PCO by inhibition of EMT, ECM deposition, and migration of LECs.

**Methods:** The antibody and inhibitors were tethered to the surface of polydimethylsiloxane (PDMS), as a model lens material via a polyethylene glycol (PEG) spacer using a method previously established in our lab. The surfaces were characterized using ATR-FTIR, contact angles, XPS, and TOF-SIMS. Morphological properties were measured by profilometry and SEM. Human lens epithelial cell lines HLE-B3 and FHL 124 interactions with modified surfaces including the production of fibroblast marker α-SMA, ECM components fibronectin and laminin, and E-cadherin shedding were measured.

**Results:** Surface modifications were confirmed by water contact angles and the presence of representative peaks in the ATR-FTIR, XPS, and TOF-SIMS. Cell viability measurement by MTT assay indicated more cell proliferation but less α-SMA and ECM components production in the presence of anti-TGF-β2 antibody (K16) compared to other inhibitors on the surface. However, tethering the inhibitors to the surface of PDMS led to decreased fibronectin and laminin deposition in the presence of exogenous TGF-β2.

**Conclusion:** Introduction of anti-TGF-β2 antibody and MMP inhibitors including sulfadiazine and TIMP-3 to the surface of PDMS as a model IOL reduced the production of α-SMA, fibronectin, and laminin by human LECs HLE-B3 and FHL 124 stimulated by TGF-β2. These modifications could be a potential indication for prevention of PCO.

Support: NSERC

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4574 - D444
Evaluation of the Viability of THP-1 Derived Macrophages Using Ethidium Homodimer-1, Calcein, and AlamarBlue to Determine the Biocompatibility of Intraocular Lens Materials
D.J. McCannan1, A.K. Weeks1, M.B. Gorbet2,3.
1Optometry, 2Systems Design Engineering, University of Waterloo, Waterloo, Ontario, ON, Canada; 3Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, ON, Canada.

Purpose: Macrophage adhesion to intraocular lens (IOL) materials may play a role in the biocompatibility of IOL. An in vitro model was developed using silicone and novel IOL materials to evaluate the viability of THP-1 derived macrophages on the materials and after exposure to varying concentrations of zinc diethylthiocarbamate (ZDEC) and benzalkonium chloride (BAK).

Methods: THP-1 derived macrophages were cultured onto HEMA and silicone hydrogels containing crosslinked hyaluronic acid (HA) and to a silicone film control biocompatible material deposited with known inflammatory chemical ZDEC (0.01%-0.1%).

After incubation in RPMI medium with 10% serum, adhered macrophages were evaluated for viability. Macrophages were also exposed to BAK at 0.001 to 0.1%. Using confocal microscopy, the number of live and dead cells adherent to the biomaterial was determined by imaging for calcein and ethidium homodimer-1 (EthD-1) fluorescence.

The cell metabolic activity was determined using alamarBlue. The degree of damage to cellular membranes was assessed using EthD-1 and the LDH assay.

Results: The adhered cells to the ZDEC containing biomaterial stained for EthD-1 indicating that all of the attached cells had damaged cell membranes. The majority of the cells treated with the silicone control material without ZDEC were live cells (calcine-stained cells). Cells on the HEMA and silicone hydrogels containing HA all stained with calcine, however different numbers of macrophages were observed. The degree of attachment appeared to be influenced by the molecular weight of HA. As expected, the ZDEC containing biomaterial and BAK caused a decrease the metabolic activity (p < 0.05). Compared to the EthD-1 assay and the alamarBlue test, the LDH assay was not effective in showing cell membrane damage as no dose relationship was observed.

Conclusions: This evaluation demonstrates that the viability of THP-1 derived macrophages adhered to biomaterials can reliably be assessed with the fluorescent dyes EthD-1, calcine and alamarBlue. Further investigations are underway to assess how HA-containing hydrogels may improve IOL biocompatibility by reducing interactions with macrophage.

CR: D.J. McCanna, None; A.K. Weeks, None; M.B. Gorbet, None.
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4573 - D443
Monovision and Asphericity With Light Adjustable Intraocular Lenses
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Purpose: To produce and evaluate monovision and aspheric extended depth of focus, in patients implanted bilaterally with light adjustable intraocular lenses (LAL).

Methods: Thirteen cataract patients were implanted bilaterally with LALs (Calhoun Vision, Pasadena, USA). Two weeks after surgery, the lenses were irrigated through the cornea with the appropriate spatial intensity profile to correct the patient's astigmatism and to leave the desired value of defocus. The surgery and the light treatments were performed first in one eye (the one set for near zero defocus) and a few months later on the fellow eye (the one set for mild myopia). Five of the patients were also treated with aspheric light adjustments to induce additional depth of focus. Wavefront-guided refraction was determined for every eye during the entire adjustment process. Visual acuity (VA) was measured using a computer-assisted procedure both monocularly (each eye separately) and binocularly.

All the VA measurements were performed for letters projected on a microdisplay placed at 10 m, 60 cm, 40 cm and 30 cm.

Results: In the eight patients treated for pure monovision the eye intended for far vision displayed a residual defocus between -0.75 D and 0.3 and the eyes intended for near vision were between -1.75 D and -2.25 D. For the distance eye, the monocular uncorrected distance VA was on average 0.9 for far, decreasing to around 0.5 at 30 cm. The eyes targeted for near vision had an average distance VA of 0.35 increasing to 0.8 for 40 cm. The measured binocular VA was usually around 1.0 for 4 m, 10 m, 40 cm and 10 cm for ZDEC. At the end of the treatments process, every patient was spectacle independent and did not report any adverse effect. For the five subjects treated with aspheric adjustments, spherical aberration was around -0.1 microns for a 4-mm pupil diameter. The eyes of these subjects intended for near vision had a residual defocus between -0.5 and -0.1D with an average VA of 0.8 for both 10 m and 40 cm.

Conclusions: The use of the LALs as an advanced and fully customized new approach to perform monovision in cataract patients was demonstrated. The LALs provide the ability to effectively set the final, desired refraction for each eye and can be reversed to emmetropia, in the event it is poorly tolerated by the patient. This approach offers a significantly improved effect, which increase depth of focus may improve monovision performance.

Support: Calhoun Vision, Pasadena, USA, “Ministerio de Educación y Ciencia”, Spain (grant gr-FIS2007-64765) and “Fundación Séneca”, Murcia, Spain (grant grant 045248/GERM/08).

4576 - D446
Comparison of Heidelberg Retinal Tomography Image Quality in Clear Lenses vs. Monofocal IOLs vs. Multifocal IOLs
Ophthalmology, Ohio State University College of Medicine, Columbus, OH.

Purpose: Heidelberg Retinal Tomography is currently used routinely in the diagnosis and management of glaucoma. The purpose of this study was to prospectively compare the quality of HRT imaging between clear natural lenses, monofocal IOLs and newer multifocal (ReSTOR) IOLs. The ReSTOR lens utilizes apodized diffactive optics, which can be structurally represented as multifocal rings on the surface of the lens. We hypothesized that the image quality and increased variability in quality of HRT images obtained in eyes with multifocal IOLs, and 11 eyes with ReSTOR multifocal IOLs was selected for this study thus far (ultimately planning for at least 29 eyes in each group). Three HRT images were obtained from each eye in the study. The MHPSD, used to quantify image quality, was recorded for each image and averaged for each eye. The mean difference in MHPSD between each of the 3 groups of patients was then analyzed for statistical significance.

Results: The mean MHPSD calculated for the group of control eyes with clear natural lenses was 15.55, the mean MHPSD for the group of eyes with monofocal IOLs was 21.15, and the mean MHPSD for the group of eyes with ReSTOR multifocal IOLs was 29.91. The standard deviations for each of these groups were 3.89, 7.16, and 9.12 respectively. Analysis of the data thus far suggests that the differences in MHPSD between these groups are, or approach, clinical significance, with the ReSTOR multifocal group producing the highest MHPSD. The differences in standard deviation in the measurements between these groups suggests that the monofocal IOLs and multifocal IOLs groups produce more varying MHPSD numbers when compared to the clear natural lens population.

Conclusions: The results thus far suggest a trend toward a significant degradation in the quality and increased variability in quality of HRT images obtained in eyes with multifocal IOLs vs. those with clear natural lenses. It is unclear whether this finding can be completely attributed to the multifocal optics of the ReSTOR lens, as the monofocal group also showed some decrease in quality when compared to controls. While all of the images may be of sufficient quality to be used clinically, it is important that IOL choice be considered when HRT imaging may be required for glaucoma management.

CR: O.C. Kuruvilla, None; A. Horne, None; Z. Qureshi, None; P. Weber, None; C. Kelley, None; T. Mauger, None; C. Roberts, None.
Support: None.

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Nanosurface Properties of Foldable Acrylic Intraocular Lenses

Purpose: Several different proprietary crosslinked acrylic copolymers are currently used for foldable intraocular lens implants (IOLs). Although extensive clinical data are reported for these lenses, there are almost no data for their surface properties. Reported here for the first time are studies concerning the nanosurface properties of IOLs from four major manufacturers.

Methods: These studies encompass (1) aqueous wetting by contact angle measurements with a custom-built apparatus, (2) viscoelastic properties using a Hysitron Triboindenter with Triboscan software and characteristic of each lens type; some rougher and some smoother on hydration. More hydrophilic IOLs exhibit less HLE cell adhesion. Nanodentation hardness and modulus also show wide-ranging differences; the elastic modulus varying from as low as 2 MPa to as high as 112 MPa.

Conclusions: Foldable IOLs in clinical use from four different manufacturers tested were found to have significantly different surface properties. Results of this study may have value in future evaluation of clinical data for these IOLs.

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Support: University of Florida Foundation

The Variability in Residual Astigmatism After Toric Intra Ocular Lenses Implantation With Different Keratometric Measuring Methods
F.M. Aljardan, M.S. Alkhazashi, M. Khuthaila, M. Dinscape. Ophthalmology, McGill University, Montreal, QC, Canada.

Purpose: To compare the effectiveness of different keratometric measuring methods in predicting the least post-operative astigmatism with TORIC Intra Ocular Lenses.

Methods: A retrospective study (n=46 patients). Preoperative keratometry measurements using 3 different methods namely the IOL Master (Zeiss 5.0 version), Automated K Topcon (KR 8800) and Topographic K (Zeiss Atlas) were collected. The former was the keratometry of choice and the one substituted in the online TORIC IOL calculator (acrysoft) to find out the proposed IOL type, power and axis. Preoperative slit lamp Axis demarcation was and all patients had S/N/6/3 IOLs implanted with a predetermined surgical Induced Astigmatism (SIA) at 0.5 D, a standard Incision location (O: 220˚; O: 40˚). The follow up was 6 months.

Post operative UCVA, auto-refraction and complications rate was studied. Then for each patient Automated Ks and Topographic Ks were substituted in the same online TORIC IOL calculator to find out the new proposed parameters and then by back calculations and vector addition analysis were compared to the data given by the IOL Master Ks trying to find which k reading among those three methods would have resulted in the least post operative residual astigmatism.

Results: Preoperative Data: Mean Age 67 years, BCVA (20/70), Astigmatic Cylinder: Range 0.75-5.25D, mean= 1.89D, Preoperative SE (-7.13 to -1.38D).

Preoperative Keratometry: IOL Master Ks: Range: 0.42-2.9D, mean= 1.66D, Automated K's: 0.5-2.6D, mean= 1.52D, Topographic K's: 0.49-2.73D, mean= 1.58D.

Postoperative data: UCVA mean (20/25). Preoperative SE (-1 to +1.25D) mean= 0.5D, Cylinder (0.5-2.6D), mean: 1.52D, Topographic K's: (0.49-2.73D), mean=1.58D.

Preoperative Keratometry: Automated Cylinder: 0.3-1.54D, mean= 0.63D, Residual Cylinder: 0.1-1.25D, mean= 0.43D.

Substitution with Automated and Topographic K's 7 (15.2%) patients and 5 (10.9%) patients in the Topographic and Automated K's subgroups, respectively were suggested to have non-Toric lenses. By doing back calculations for each individual patient the average residual cylinder was found to be (0.1-5.0D), mean= 0.54D and (0.1-7.5D), mean= 0.78D in the Automated Ks and Topographic K's subgroups, respectively. No post-operative complications were documented.

Conclusions: TORIC IOLs resulted in excellent level of postoperative uncorrected visual acuity (UCVA) and eliminated about 66% of corneal astigmatism when IOL Master K's were. IOL Master K's resulted in the least amount of residual astigmatism when used in the TORIC IOL calculations in comparison to the automated and topographic K's.

CR: F.M. Aljardan, None; M.S. Alkhazashi, None; M. Khuthaila, None; M. Dinscape, None. Support: None
4581 - D651
A Novel Method for the Detection and Quantification of Dideoxyosone Intermediates of AGEs in Human Lens Proteins
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Purpose: Dideoxyosones are intermediates in the synthesis of advanced glycation end products (AGEs) such as pentosidine and glucosepane. Although the formation of pentosidine and glucosepane in the human lens has been firmly established, the formation of dideoxyosone has not been demonstrated. The purpose of this study was to develop a novel approach to detect and quantify dideoxyosones in lens proteins.

Methods: We synthesized a dideoxyosone trap, 3,4-diamino-N-[5-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoyl]laminopropyl]benzamide (BDAB) (structure shown below). BDAB was added at 300 μM concentration to one half of the freshly isolated lens and the tissue was homogenized add 1.0 ml 0.1M sodium phosphate buffer, pH 7.4 containing 0.1 M NaCl and 1 mM EDTA. The other half was homogenized similarly without BDAB. Lens homogenate was then lyophilized. The lyophilized sample was sonicated in HEPES buffer containing 1% Triton X-100. The sample was then passed through SDR-Hyper-D resin and the resulting protein was coated onto ELISA plate wells at 5 μg/well. The wells were blocked with 5% non-fat dry milk, incubated with avidin-conjugated to horseradish peroxidase (HRP) followed by incubation with a substrate for HRP. BSA (1 mg/ml) was glycinated with 1 mM ribose in the presence of 10% DMSO for 24 h. Results: BSA incubated with ribose and BDAB showed high levels of dideoxyosones. Controls in which ribose or BDAB was omitted during incubation showed either no reaction or a 5-10 fold decrease in reaction. BDAB added cataractous human lens but not the controls (no BDAB added) showed high levels of dideoxyosones. The levels were 4.7, 4.1, 7.6 and 9 units per 5 μg protein in cataractous lenses from 40-50, 50-60, 60-70 and 70-85 year, respectively.

Conclusions: Dideoxyosone intermediates are present in human lens proteins and they could be major intermediates for the synthesis protein crosslinking AGEs in cataractous lenses.

CR: M.D. Linetsky, None; K. Johar, None; S. Padmanabha, None; T. Parmar, None; A.R. Vasavada, None; R.H. Nagaraj, None.
Support: NIH grants R01EY-06219, R01EY-09912 and P30EY-11373, RBP and OLERF

4581 - D652
The Biochemical Alterations in Lenses of Thioltransferase (TTase) Knockout Mice
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Purpose: Thioltransferase knockout (TTase KO) model has been established in this laboratory and showed cataract development sooner than the wild type (WT). The purpose of this study is to examine the relationship of morphological changes with the biochemical alterations in the lenses of TTase KO and WT mice as a function of age.

Methods: Mice of matching age of WT (0.5, 4.5, 9.2 ml) and KO (1.3, 4.6, 8.9 ml) were examined for lens opacity with portable slit lamp and Fundus camera. Each lens was homogenized in lysis buffer and processed for measurement of glutathione (GSH) level by DTNB colorimetric method and examination of protein-GSH mixed disulfides (PSSG) formation by Western blot analysis using an anti-GSH specific antibody. Dethiolation of lens proteins was carried out using either DTT or purified human recombinant TTase.

Results: The slit lamp examination and Fundus camera showed a gradual cataract development in both eyes of WT and KO mice as a function of age. Nuclear cataract then appeared to appear sooner in KO mice (4 ml) compared with WT mice (9 ml). The opacity in both lenses of the 9 months-old KO mouse also covered cortical and subcapsular regions. Western blot analysis showed that PSSG gradually increased with age and corroborated with the severity of opacity. PSSG was more abundant in the KO group than the WT group. These GSH-conjugated proteins could be eliminated when the lens homogenate was either treated with DTT or TTase. Interestingly GSH levels in these lenses remained relatively unchanged.

Conclusions: The present results showed that detection of TTase gene in the mouse could lead to an early age-dependent cataract formation. The spontaneously accumulated PSSG in these lenses appeared to link directly to lens opacity.

CR: J. Zhang, None; K.-Y. Xing, None; M.F. Lou, None.
Support: NIH Grant EY10955

4583 - D653
Protein Profile Changes in Monkey Lenses Due to Aging and Soy-Diet
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Purpose: To analyze the profiles of water soluble (WS) and water insoluble (WI) lens proteins of young and old monkeys (Macaca fascicularis) fed soy-rich and soy-deficient diets.

Methods: The monkey lenses were divided into the following four experimental groups: (i) 9-year-old fed a soy-free diet for 30 months, named YC; (ii) young on soy-diet (6- to 9-year-old fed soy 2 mg/ml for 30 months, named YS); (iii) old control (15-year-old or older fed a soy-free diet for 30 months, named OC); and (iv) old on soy-diet (15-year-old or older fed soy 2 mg/ml for 30 months, named OS). Two-Dimensional Fluorescence Difference Gel Electrophoresis (2D-DIGE) was used to investigate the statistically significant changes in protein abundance in the WS- and WI-protein fractions of each group, and their identity and post-translational modifications (PTMs) were determined by Q-TRAP mass spectrometric method. Multi-angle light scattering (MALS) was used to determine the molar mass of the WS-proteins of the four experimental groups.

Results: Lenses from the YS group exhibited relatively greater levels of WI-proteins compared to lenses from YC, OC, and OS groups. MALS results showed a higher molar mass range of WS- and WI-proteins in WS lens compared to YC lenses, but a similar molar mass range to those from OC and OS lenses. The 2-D DIGE analysis of WS- and WI-protein profiles showed statistically significant differences between the YC and YS groups, but such differences were not found between the profiles of lenses from OC and OS groups. Additionally, even matched spots with statistically similar amounts of proteins contained varied crystallins and PTMs. Some PTMs such as oxidation, methylation, and deamidation of crystallins increased with aging, and these also increased in lenses of the YS group compared to the YC group.

Conclusions: The proteome of monkey lenses compared to inflammatory and immune response, and transport.

CR: J.M. Chaves, None; R. Gupta, None; K. Srivastava, None; A.K. Gill, None; L.S. Wilson, None; O.P. Srivastava, None.
Support: NIH-EY16400 and EyeSight Foundation of Alabama

4584 - D654
Protein Profiling of Human Aqueous Humor by Mass Spectrometry
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Purpose: We report the first detailed and comparative quantitative analysis of the protein content of aqueous humour from human eyes with cataract by mass spectrometry. In the present study, iTRAQ labelling and LCMS analysis, the approach is layered with quantitative proteomics using the iTRAQ methodology.

Methods: Aqueous humour samples from ten clinically-matched cataract patients were collected, and pairs of patient material were pooled, mixed and divided equally into three aliquots. Proteins from one of the pair-wise pooled samples were separated by one-dimensional gel electrophoresis, followed by tryptic in situ digestion and LCMS on a hybrid LTQ Orbitrap XL mass spectrometer. Another third was digested in solution and separated by reverse-phased reversed-phase two-dimensional LCMS; and the final third was labelled with 4-plex iTRAQ reagents and separated by 2D-LCMS.

Results: A total of 198 protein groups were identified across the entire study. Relative protein quantitation with iTRAQ revealed that 60% of the proteins had an approximate equimolar distribution between 3 of the 4 labelled samples, indicating minimal variation between the control cataract samples. The identified proteins were categorised by gene ontology database entries with respect to cellular compartmentalisation, molecular function, and biological processes. One third of the proteins were annotated as extracellular. The major molecular functions of the proteins in aqueous humour are binding and inhibition of proteolytic activity. Complementary to molecular function, the predominant biological processes for the proteins in aqueous humour are assigned to inflammatory and immune response, and transport.

Conclusions: Our results provide an overview of the proteins present in AH of cataractous eyes. In summary, the 198 unique protein groups identified via the combined methods are involved in diverse biological processes and function such as binding, inhibition of proteolytic activity, transport, immune, and inflammatory response. This study is an initial step toward establishing the foundations for future proteomic investigations.

CR: M. Funk, None; K.L. Bennett, None; A. Pollrez, None; M. Planjansky, None; S. Sacu, None; C. Ubaida Mohien, None; A. Müller, None; J. Colinge, None; G. Superti-Furga, None; U. Schmidt-Erfurth, None.
Support: None

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**4585 - D655**

Proteomic Analysis of Lens Soluble Fraction in Different Types of Cataracts

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**Purpose:** To analyze the proteomic changes of the lens in patients with age-related cataract (AR), posterior subcapsular cataract (PS), pseudoexfoliative cataract (PSX), and cataract in Retinitis pigmentosa (RP) and to compare with a control clear lens group (CL).

**Methods:** A total of 42 lens samples were collected from each individual patients (AR=11; PS=9; PSX=9; RP=5 and CL=8). The samples of lenses were obtained after phacoemulsification. Immediately after their collection all samples were stored at -80°C until the analyses. To analyze the proteomics of the lenses, the samples were fractionated into two phases according to the solubility. Quantification of protein concentration was determined using EZQ fluorescence protein Quantitation kit (Invitrogen). 80 µg of soluble fraction was separated in the first dimension by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 15% polyacrylamide gels. The gel images were analyzed using Progenesis SameSpots software. Those spots with a significant fold change were automatically cut out from the gels and the corresponding proteins were identified by MALDI-TOF.

**Results:** 148 spots of proteins were detected in the study, of which 54 have been identified, corresponding with 10 individual proteins and multiple isoforms. The analysis of the expression of proteins in the different types of cataracts shows a different expression profile. Plotting the significance data in a multidimensional space, by principal component analysis (PCA) indicated that several protein spots enable a perfect separation between the groups (AR, PS, PSX, RP) comparing to the control group (CL). This clustering is also possible within the pathologic groups to different types of cataracts. Furthermore, the MALDI-TOF MS results indicate that the proteins which are more involved in that differentiation between groups were Actin, α-crystallin B2, β-crystallin B1, β-crystallin A3, β-crystallin S according to their differential protein expression.

**Conclusions:** The results of the present study show a difference in expression pattern of the soluble proteins related with the different types of cataract. MALDI-TOF identification of several spots as the same protein suggests that the differences between groups could be due, not only to a change in the protein expression profile, but also to proteolytic changes in the proteins.

CR: C. Irigoyen, None; J. Soria, None; A. Acera, None; T.M. Suarez-Cortes, None; J. Mendicute, None.

Support: None.

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**4587 - D657**

Mapping the Lens Metallome in Normal Aging and Alzheimer’s Disease by Laser Ablation Sector Field Inductively Coupled Mass Spectrometry (LA-SS-ICP-MS)

N.F. Casey, J.A. Moncaster, O. Mineva, M. Burton, A. Fraine, S. Sarangi, L.E. Goldstein

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**Purpose:** We previously identified amyloid β (Aβ) deposition, amyloid pathology, and co-localizing subsequeal anterior subcapsular cataract in Alzheimer’s disease (AD). Goldstein et al. [68], and Simpanya et al. [69] demonstrated the involvement of metals in the aging of the human lens. In AD lenses, Aβ aggregates as hetero-oligomeric complexes that localize to the cytosol of supranuclear fiber cells. Emerging evidence indicates that metals bind to and promote Aβ oligomerization, determining the spatial distribution of trace metals in lens and brain will facilitate elucidation of pathogenic metalprotein interactions in affected tissues. Here we utilize state-of-the-art laser ablation sector field inductively coupled mass spectrometry (LA-SS-ICP-MS) to generate high-resolution ultra-trace elemental and isotopic distribution for Cu, Zn, Ca, Mg, Na, P, Rb, C and S in aged human lens extracts of both normal and AD patient lenses. Here we compared the uptake of the normal (N) to AD (A) groups (n=10/group) with and without AD pathology as described earlier (Goldstein et al. 52,2008). As a preamble to the topical application of NC1 as a drug applied to the human eye, we have compared the uptake of NC1 in normal and acetyl-cysteine (NAC) in rabbit lens in vitro and in vivo, as well as the ability of N1 to block ascorbylation reactions when applied topically to hSVCT2 mouse model of lenticular aging.

**Results:** The results indicate aging of the human lens is associated with trancification and truncification of lens α- and β-crystallins. These changes may contribute to water-insolubilization since they were more pronounced in this fraction. However, truncation of α-crystallin, whose truncation results in crystallin insolubilization through loss of chaperone activity. The 2-D maps created will be useful for comparison with protein maps of human lenses and cataract related changes and for comparison with other species.

CR: N.F. Casey, None; L.L. David, None; L.J. Robertson, None; P.A. Wilmarth, None; F.J. Gobin, None.

Support: NIH Grants EY02027 and EY014803 (JCG), EY007755 and EY010572 (LID)

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**4586 - D656**

Age-Related Protein Modifications of Guinea Pig (Cavia porcellus) Lens Crystallins Analyzed by Two Dimensional Gel Electrophoresis and Mass Spectrometry

M.F. Simpanya, L.L. David, L.J. Robertson, P.A. Wilmarth, F.J. Gobin

Eye Research Institute, Oakland University; Rochester, MI; Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR.

**Purpose:** To identify age-related modifications (i.e. protein truncations, changes in isoelectric points and insolubilization) of guinea pig lens crystallins, and to produce two dimensional electrophoresis (2-DE) maps of cortical and nuclear water soluble intact lens protein of young and old pig lenses.

**Methods:** Lenses from 2.5-month and 24-month-old guinea pig were dissected into nuclear and cortical regions, and WI and W2 fractions isolated by centrifugation, and proteins separated by 2-D gels. Protein spots on 2-D gels were excised, digested and tryptic peptides analyzed by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). Spots not able to be identified by MALDI-MS were analyzed by LC-MS/MS.

**Results:** Beyond the high abundance of α-crystallin, the crystallin composition of the guinea pig was unusual in that there was an abundance of β3-crystallin. Similar to other mammals, β3-crystallin was converted to partially truncated forms that were more abundant in the WI fraction in the nucleus of both young and old animals. Multiple truncated forms of β1-crystallin with a wide range of ps were also observed in all fractions and regions of lenses. However, β1-crystallin was fractionation was observed, the second dimension was carried out using SDS-PAGE in 15% polyacrylamide gels. The gel images were analyzed using Progenesis SameSpots software. Those spots with a significant fold change were automatically cut out from the gels and the corresponding proteins were identified by MALDI-TOF.

**Conclusions:** The results indicate aging of the human lens is associated with truncification and truncification of lens α- and β-crystallins. These changes may contribute to water-insolubilization since they were more pronounced in this fraction. However, truncation of α-crystallin, whose truncation results in crystallin insolubilization through loss of chaperone activity. The 2-D maps created will be useful for comparison with protein maps of human lenses and cataract related changes and for comparison with other species.

CR: M.F. Simpanya, None; L.L. David, None; L.J. Robertson, None; P.A. Wilmarth, None; F.J. Gobin, None.

Support: NIH Grants EY02027 and EY014803 (JCG), EY007755 and EY010572 (LID)
Organizing Section: LE

4589 - D659

A Numerical Biomechanical Model of Accommodation as Part of a General Biomechanical Model of the Eye

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Purpose: As the choice of adequate means for intravital observation and quantitative evaluation of accommodation physiology is limited, its biomechanical modeling is advisable. Purpose: numerical modeling of accommodation within the deformation model of human eye; a study of the impact of biomechanical parameters of certain tissues of the eye on its accommodative ability.

Methods: The finite element method (ANSYS) has been applied. A nonlinear 3D problem was assumed which took account of the liquid, which was being modeled statically. The parameters that determined accommodative ability of the eye included accommodation volume as well as energy used to shift the gaze from the near to the distant target and vice versa.

Results: The numerical biomechanical model of the eye includes the crystalline lens (containing the nucleus and the capsule), ciliary body, zonular fibers, iris, limbus, cornea, sclera, extracapsular muscles (including the eye string), optic nerve, vitreous, choroid, retina. A series of analyses have been performed to observe processes of accommodation and eyeball rotation in the eye socket with various values of mechanical properties of eye tissues. Optical power and energy required for crystalline lens deformation under the simulated processes have been evaluated (Fig.1,2).

Conclusions: Accommodation volume and energy used depend on the value of the force created by active contraction of the ciliary muscles, mechanical properties (rigidity) of the crystalline lens content, the rigidity ratios of lens capsule/cortex/nucleus, as well as biomechanical properties of the sclera and extracapsular muscles. It is shown that the anterior-posterior axis of the eye may increase by up to 0.5 mm with the contraction of the extracapsular muscles. Numerical modeling of accommodation within the complete biomechanical model of human eye shows that it agrees with Helmholtz accommodation theory.

Support: None.

4591 - D661

Increase in Lens Thickness as Measured by A-Scan Ultrasonography in an Older African-American Population


Purpose: We create dynamic computer models to recreate anatomical relationships for lens aging in an older population. Purpose: to determine the structural changes that occur in the lens with age.

Methods: A numerical model of the human eye was created to simulate aging processes, with a focus on age-related changes in the lens. The model was validated using data from a previous study on lens thickness.

Results: The model predicted an increase in lens thickness of 0.1 mm per decade, which is consistent with the observed increase in lens thickness measured in the study.

Conclusions: The numerical model accurately predicts the increase in lens thickness with age, providing a useful tool for understanding the impact of aging on the lens.

Support: NIH K23 EY01409, core grant EY01894, EY01628, EY05722, Research to Prevent Blindness (RPB) SYBil H. Harrington Scholar Award

4592 - D662

Quantitative Measurement of Crystalline Lens Opacification

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Purpose: To identify a quantitative measure of crystalline lens light characteristics that may provide insight into cataract progression over time.

Methods: Archived fundus images from 105 phakic and 104 pseudophakic eyes were analyzed for their spectral characteristics using Adobe Photoshop® CS2. Eyes with cortical or posterior subcapsular cataracts and capsular opacities were excluded.

Results: Of the ten spectral characteristics analyzed, blue light absorption showed the greatest difference between phakic and pseudophakic eyes when measured in the optic cup, disc, and vessel. Mean blue value in the cup of phakic patients was significantly less than that of pseudophakic patients (p=0.001). No difference was noted when comparing clear phakic and pseudophakic lenses (p=0.16). Analysis of the spectral characteristics in blue showed that patients with 0+NS (91.5±44 vs 105.6±36.5; p=0.01). Overall, phakic patients without opacification had blue values greater than phakic patients with opacification (91.5±44 vs 79.7±33.7; p=0.01). No difference was noted when comparing pseudophakic patients.

Conclusions: Our analysis reveals that there are quantifiable differences in spectral light characteristics between phakic and pseudophakic eyes. Blue light absorption was shown to be inversely associated with increasing lens opacification. Major limitations of this study were the subjective grading of crystalline lens opacity and variable use of camera flash, more accurate identification and revised imaging protocol may improve the spectral correlations. These findings may aid in the development of an objective cataract grading scale to improve correlation with visual symptoms, clinical diagnosis, and assessment of longitudinal progression.


CR: W. Huang, None; J. Qiu, None; I. Navarro, None; P. Gonzalez, None; P. Challa, None.
Support: NIH K23 EY01409, core grant EY01894, EY01628, EY05722, Research to Prevent Blindness (RPB) SYbil H. Harrington Scholar Award

FOXO Protein Expression is Down-Regulated With Aging in the Lens


Purpose: Cataracts is a leading cause of age-related blindness worldwide. With aging, accumulation of oxidized lens components and decreased efficiency of repair mechanisms can contribute to the development of lens-opacities i.e. cataracts. FOXO (forkhead box O) transcription factors play key roles in cellular resistance to oxidative stress. In response to oxidative stress, FOXO activity is regulated primarily through activation of its protein levels, subcellular localization and post-translational modifications. We hypothesized that FOXO expression in the lens is altered with aging. Purpose: to study the change of FOXO activation in the aging lens.

Methods: Lenses were collected from DBA/2 mice at 3, and 18 months old. The 18 months old mice had advanced nuclear cataract formation. Immunoblot analysis of FOXO1, FOXO3a as well as the phosphorylated forms was performed. Alpha-tubulin was used as a loading control and all values were quantified using densitometry. Statistical analysis was performed using a 2-way analysis of variance and significance was assessed at the 0.05 level.

Results: FOXO1, FOXO3a levels were decreased significantly (p<0.05) at 54% and 76% respectively in the aging lens. Also, a significant decrease of both phosphorylated forms of FOXO1 and FOXO3a at 41% and 78% respectively were observed in the aging lens.

Conclusions: FOXO1, FOXO3a as well as their phosphorylated forms are down regulated with age in the rodent lens. FOXO proteins play essential roles in the cellular response to oxidative stress and phylogenetic program and are involved in the aging of lens.

Support: None; J.R. Kuszak, None; D.W. Huang, None; I. Navarro, None; P. Gonzalez, None; P. Challa, None; C; MacuSight – REDIARC Fundus Reading, C; American Academy of Ophthalmology, C; Therapeutic Nanoparticle and Molecular Imaging, C; Digital Healthcare, Inc., C; Plough – REDIARC Fundus Reading, C; VRT – Vitreo Retinal Technologies – REDIARC, C; Neurotech – REDIARC Fundus Reading, C; Lux Bio – REDIARC Fundus Reading, C; A Basic Science and Treatment Fund, C; MacuSight – REDIARC Fundus Reading.
219. Functional Retinal Imaging

**Organizing Section: LE**

Monday, May 4, 8:30 AM - 10:15 AM Hall B/C Poster Session Program Number/Board # Range: 1382 - 1406 / B121 - B145

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**Palla3. Zablocki model. HSIR/ AKR1B10 has little impact on lens metabolism and transparency even null mice failed to accumulate sorbitol and maintained normal levels of glutathione.**

**Conclusion:**

- Glucose (27.5 mM), lenses from HAR/ AKR1B1 accumulated large amounts of sorbitol and lost approximately half of normal glutathione levels after 3 days; these changes were maintained for at least 6 weeks following induction of experimental diabetes. Virtually no difference in lens transparency was observed of either HAR/ AKR1B1 or HSIR/ AKR1B10 was monitored by slit lamp ophthalmoscopy for at least 6 weeks following induction of experimental diabetes (streptozocin). Digital images were examined by masked reviewers to score for the presence and extent of cataract formation and progression were observed in 10/12 (83%) of the dogs receiving placebo. In contrast, after 12 months cataract formation in the Kinostat® group I, 19.4±4.5 in group II (p<0.01), 28.5±6.5 in group III (p<0.01), and 6.2±2.3 in group IV (p<0.01). All measurements showed high correlation between lens transparency and lens densitometry.

**Conclusions:**

- We revealed high positive correlation between lens transparency (natural or artificial lens) and lens densitometry. No correlation was found between lens transparency and lens densitometry between patients of different age groups.

**Support:**

- None

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**4596 - D666**

**Kinostat® Reduces the Clinical Onset and Development of Cataracts in Diabetic Dogs**

- 1Pharmaceutical Sciences, Univ of Nebraska Medical Center, Omaha, NE; 2Therapeutic Vision Inc., Omaha, NE; 3Ophthalmology, MedVet Medical Center for Pets, Worthington, OH; 4All Animal Eye Clinic, Cincinnati, OH.

**Purpose:**

- Diabetes mellitus is characterized by the development of cataracts which generally occurs within 1 year of the time of diagnosis. The purpose of this study was to investigate whether the topical administration of the aldose reductase inhibitor Kinostat® can ameliorate the onset or progression of cataracts in naturally occurring diabetic dogs.

**Methods:**

- Forty newly diagnosed diabetic dogs with minimal lens changes were enrolled in a prospective, masked pilot study. Dogs were randomly assigned a coded vial containing either Kinostat® or vehicle (placebo), with the contents of the vial (drug or placebo) masked from the examiner. Twenty-eight dogs received KinostatTM, and 12 dogs received placebo. Owners were instructed to administer the agent OU TID for 1 year, and were instructed to record each time of administration in order to ensure compliance. Complete ophthalmic examinations were performed prior to enrolling in the study, and then at 1, 2, 3, 6, 9, and 12 months and approximately 4-6 month intervals thereafter.

**Results:**

- Cataract formation and progression were observed in 10/12 (83%) of the dogs receiving placebo. In contrast, after 12 months cataract formation in the Kinostat® group was significantly (p = 0.0016) inhibited with 15/28 (53.6%) of dogs receiving Kinostat® not showing evidence of cataract development. Of the 20 Kinostat® treated dogs remaining on study at time periods ranging from 14 to 22 months, 13 have no lens changes, 6 have cortical vacuoles and 1 has a cortical opacity. This is significantly different (p = 0.0001) from the 7 remaining placebo dogs where only 1 has no lens changes.

**Conclusions:**

- Topical Kinostat® is beneficial for up to 2.5 years in reducing the onset and/or progression of cataracts in dogs with diabetes mellitus.

**CR:**

- H. Kawada, None; M. Wyman, None; T. Webb, None; D. Bras, None; K. L. Ketring, None; P.F. Kador, None.

**Support:**

- None

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**Conclusions:**

- Aldose reductase is primarily responsible for diabetes-related sorbitol accumulation and oxidative stress associated with cataract formation in our mouse model. HSIR/ HKR1B10 has little impact on lens metabolism and transparency even following diabetes induction.

**CR:**

- J. M. Petrash, None; A. Varma, None; T.M. Harter, None; P.A. Ruzyczki, None; G.J. Zabolocki, None; B.G. Reddy, None; S. Palla, None.

**Support:**

- NIH Grant EY03856 (UCDenver); D3T (Hyderabad)

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**4594 - D664**

**Lens Densitometry Measurements by “Pentacam”**


**Purpose:**

- To evaluate lens densitometry in patients with transparent lens and patients before and after cataract surgery.

**Methods:**

- We examined a series of 138 eyes of 69 patients, aged from 18 to 76 years. Patients were divided into following groups: group I - 48 eyes of 24 patients with transparent lens (control); group II - 30 eyes of 15 patients with mild cataract; group III - 28 eyes of 14 patients with premature cataract; and group IV - 32 eyes of 16 patients after cataract surgery with different types of intraocular lens. Lens densitometry was measured using non-contact rotating Scheimpflug camera “Pentacam” (Oculus).

**Results:**

- Results of groups II, II, and IV were compared to group I, using “Microsoft Excel” and “Statistica” software.

**Results:**

- Lens densitometry measured by “Pentacam” was 12.6±2.2 standard units in group I, 34.4±4.5 in group II (p<0.01), 28.5±6.5 in group III (p<0.01), and 6.2±2.3 in group IV (p<0.01). All measurements showed high correlation between lens transparency and lens densitometry. No correlation was found between lens transparency and lens densitometry between patients of different age groups.

**Support:**

- None

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4593 - D665

**Lens Phenotypes Associated With Transgenic Expression of Human Aldo-Keto Reductases**

- 1Ophthalmology/Rocky Mt Lions Eye Inst, Univ of Colorado Denver, Aurora, CO; 2Ophthalmology & Visual Sciences, Washington University, St. Louis, MO; 3Biochemistry, National Institute of Nutrition, Hyderabad, India.

**Purpose:**

- To develop an animal model for testing and validating inhibitors of diabetic eye disease, we produced lines of transgenic mice that over-express either human aldose reductase (HAR, AKR1B1) or human small intestine reductase (HSIR, AKR1B10).

**Methods:**

- Lens transparency in transgenic lines that express roughly equivalent levels of either HAR/AKR1B1 or HSIR/AKR1B10 was monitored by slit lamp ophthalmoscopy for at least 6 weeks following induction of experimental diabetes (streptozocin). Digital images were examined by masked reviewers to score for the presence and extent of lens opacities. Effects of hyperglycemia were evaluated following organ culture of lenses in the presence of high glucose with or without an aldose reductase inhibitor. Sorbitol and glutathione levels were measured enzymatically.

**Results:**

- Presence and severity of cataract was significantly higher in HAR/ AKR1B1 transgenic as compared to nontransgenic control mice after induction of experimental diabetes. Virtually no difference in lens transparency was observed between HSIR/AKR1B10 transgenic and nontransgenic controls with or without diabetes induction. However, an anterior lens defect was observed in HSIR/AKR1B10 after long term (6 months) of diabetes. When organ cultured in the presence of high glucose (27.5 mM), lenses from HAR/AKR1B1 accumulated large amounts of sorbitol and lost approximately half of normal glutathione levels after 3 days; these changes were completely prevented by sorbinil. In contrast, lenses from HSIR/AKR1B10 and AR null mice failed to accumulate sorbitol and maintained normal levels of glutathione.

**Conclusion:**

- Aldose reductase is primarily responsible for diabetes-related sorbitol accumulation and oxidative stress associated with cataract formation in our mouse model. HSIR/AKR1B10 has little impact on lens metabolism and transparency even following diabetes induction.

**CR:**

- J. M. Petrash, None; A. Varma, None; T.M. Harter, None; P.A. Ruzyczki, None; G.J. Zabolocki, None; B.G. Reddy, None; S. Palla, None.

**Support:**

- NIH Grant EY03856 (UCDenver); D3T (Hyderabad)
4597 - D667
Expression of Metallothionein and Crystallins in a Rat Model for Diabetic Cataract
T. Holm, L. Johnson, L. Kessel. Ophthalmology, Gloustrup Hospital, Copenhagen, Denmark.

Purpose: Age-related cataract is defined as an opacification of the lens caused by alterations in the lens proteins. Metallothionein (MT) is known to increase at the transcriptional level as a response to oxidative stress and developing cataract. In this study we have determined similar changes to expression of α-A and γ-D-crystallin throughout the lens.

Methods: Protein distribution was visualized using immunohistochemistry (IHC) on frozen section of the central region of the lens. Lenses were obtained from several ZDF rats with and without diabetic cataracts, fixed in 4% paraformaldehyde over night, and stained with antibodies for MT, α-A, β-JBI- and γ-D-crystallin.

Results: Increased MT expression was found in the epithelium of cataract lenses, with no notable presence in non-cataract lenses. Expression of γ-D-crystallin was likewise increased in the epithelium as well as throughout the lens fibers in cataractous lenses compared to controls. A smaller increase was observed for α-A-crystallin in the epithelium and outer cortex regions, whereas no change was observed with β-JBI-crystallin.

Conclusion: Diabetic cataract in rat lenses is marked by increased expression of MT in the epithelial cells, γ-D-crystallin and partly α-A-crystallin expression in the epithelium and lens fibers.

CR: T. Holm, None; L. Johnson, None; L. Kessel, None.
Support: None

4598 - D668
Coordinate Alzheimer Aβ Pathology in Lens and Brain in Diabetes: Non-Invasive Laser-Based Detection of Aβ Biomarkers in Lenses
P.H. Frederikse1A, Y. Feng1B, C.L. Bitel1A. Pharmacology Physiology & Rutgers/UMDNJ Int. Neurosciences, Pharmacology, UMD New Jersey Medical School, Newark, NJ.

Purpose: Alzheimer Aβ pathology is mechanistically linked with diabetes and insulin resistance. Recently, we showed that neuronal and Alzheimer related vesicle transport proteins are extensively shared in the lens, and that basic transcriptional and post-transcriptional regulation that governs neurogenesis also uniquely occurs in lens. Here, we test the idea that as a result, coordinate Aβ pathology occurs in lens and brain when we apply system-wide stress in the form of diabetes and hyperglycemia. We also developed a laser-based instrument to non-invasively detect and measure Aβ biomarkers in the intact lens.

Methods: Brain and lens from rabbits rendered diabetic with alloxan for 16 wks were examined using in situ, ELISA and laser spectrometry Aβ detection methods. Insulin receptor and Aβ antibodies against monomeric and higher order Aβ moieties were used.

Results: Widespread Aβ pathology was detected with antibodies against monomeric and higher order Aβ structures in brain, and lenses. Aβ plaques were widespread in hippocampus and cortex and Aβ in lenses accumulated primarily in the periphery. Separate quantitative ELISA assays detected >4-fold increase in Aβ ending at amino acid 40 and ending at 42 in diabetic lens, hippocampus and cortex. Laser spectrometry measured 4-fold increase in Aβ in intact lenses, matching ELISA determinations.

Conclusions: Substantial Aβ pathology was coordinately produced in brain and lens in wild-type diabetic rabbits after 16 weeks. Non-invasive laser spectrometry measurements of Aβ biomarkers in lens matched levels determined in vitro with ELISA methods and agree with in situ results. Our 16 week “end-point” study provided strong evidence consistent with our hypothesis that corresponding Aβ pathology and Aβ accumulation coordinately occurs in brain and lenses, which can be detected and quantified spectrophotometrically in the lens. Future studies on the kinetics and relative onset at earlier time points after inducing diabetes and hyperglycemia will determine the ability to use Aβ levels in tissue outside the brain “like a blood test” (or other fluids that have been proposed), and will determine the ability of our spectrometry instrumentation to provide useful diagnostic information about the onset and progression of Aβ pathology in brain, beginning with models of shared systemic stress in diabetes.

CR: P.H. Frederikse, UMDNJ-NJMS, P; Y. Feng, None; C.L. Bitel, None.
Support: 1R01EY015855, NJ Tech and Commercialization Fund
**4607 - D670**

PKC Phosphorylation Can Completely Eliminate AQP0 Water Permeability


**Purpose:** Investigate the effects of PKC phosphorylation on the water permeability of AQP0.

**Methods:** Xenopus oocyte water permeability assay, biAQPO, site directed mutagenesis, PKC activation, PKC inhibition, Fluorescent Resonance Energy Transfer (FRET), immunohistochemistry.

**Results:** AQP0, the major intrinsic protein of lens fiber cells, can act as a water channel, jonctional protein, or anchor protein for the cytoskeleton and promotes the formation of micro-domains in the plasma membrane. During fiber cell differentiation AQPO undergoes spatially and temporally regulated phosphorylations that could have unique physiological roles in lens fiber cells. The pattern of C-terminal phosphorylation of AQP0 creates four distinct water permeability (Pf) phenotypes: 1. Low-Pf, with no Ca2+ sensitivity; 2. Low-Pf, with 5 mM Ca2+ sensitivity; 3. Low-Pf, no Ca2+ sensitivity; 4. High-Pf, with no Ca2+ sensitivity (Kalman, K et al. JBC, 2008). In the inner cortex, Ser229 and Ser231 phosphorylation increases (Ball, L.E. et al. Biochem. 2004), and these modulated AQPOs become calcium-independent high-Pf Pf phenotype channels. Using site-directed mutagenesis, PKC activators and inhibitors, we investigated the effects of PKC phosphorylation(s) on “group-4” Ser229Asp, Ser229Asn, Ser231Asp and Ser231Asn mutants. Our data show that DAG-activated PKC inhibits the Pf of Ser229Asp mutant and probably closes the channel pore. This result reveals a fifth phenotype of regulation of AQP0 by phosphorylation.

**Conclusions:** PKC phosphorylation is an essential component of the regulation of AQP0 water permeability. Our observation suggests that there is a particular combination of C-terminus serine-phosphorylation which completely eliminates AQP0 water permeability. This suggests a greater range of Pf regulation then before observed.

**CR:** K. Kalman, None; K.L. Nemeth-Cahalan, None; D. Clemens, None; J.E. Hall, None.

**Support:** NIH EY5561

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**4602 - D672**

Gap Junctions Are Selectively Associated With Interlocking Ball-and-Sockets but Not Protrusions in the Lens

S.K. Biswas, J.E. Lee, L. Brako, W.X. Lo. Department of Neurobiology, Morehouse School of Medicine, Atlanta, GA.

**Purpose:** Ball-and-sockets and protrusions are specialized interlocking membrane domains between lens fibers of all species studied. Ball-and-sockets and protrusions resemble one another in their shape, size and surface morphology, and are traditionally believed to play a key role in maintaining fiber-to-fiber stability. Here, we evaluate the relative contribution of ball-and-sockets and protrusions possess important structural and functional differences during fiber differentiation and maturation.

**Methods:** Intact lenses of leghorn chickens and rabbits monkeys at various ages were studied with SEM, freeze-fracture TEM, freeze-fracture immunogold labeling (FRIL), and filipin cytochemistry for membrane cholesterol detection.

**Results:** SEM showed that numerous ball-and-sockets were distributed along the long and short sides of hexagonal cortical fiber cells, whereas protrusions were located along the cell corners from superficial to mature cortical regions in both species. Strikingly, by freeze-fracture TEM, we discovered the selective association of gap junctions with ball-and-sockets examined, but not with protrusions, in both embryonic and adult chicken lenses. Many ball-and-socket gap junctions protruded deeply into the neighboring cells. Similar results were found in the young and adult monkeys. A number of ball-and-sockets in the mature fibers of monkey lenses exhibited only partial occupancy of disorganized connexons, suggesting the presence of degenerating gap junctions. FRIL confirmed that both Cx46 and Cx50 antibodies were specifically observed in ball-and-sockets, but not in protrusions. While the ball-and-socket gap junctions displayed different amounts of cholesterol (i.e., cholesterol-rich vs. cholesterol-free) in different cortical regions during maturation, the protrusions contained constant high cholesterol amounts which were approximately two times higher than that of the cholesterol-rich gap junctions found in ball-and-sockets.

**Conclusions:** This study suggests that the ball-and-socket gap junctions facilitate their communicating role by protruding deeply into the neighboring fiber cells to increase membrane surface areas for cell-to-cell communication. In contrast, the protrusions may play a primary role in maintaining fiber-to-fiber stability in the lens. Thus, the ball-and-sockets and protrusions are two structurally and functionally distinct membrane domains in the lens.

**CR:** S.K. Biswas, None; J.E. Lee, None; L. Brako, None; W.X. Lo, None.

**Support:** NIH Grant EY10534
Overexpression of Cx46 Causes Proteasome-Dependent Degradation of Cx43 in Lens
L.K. Bossmann, D. Banerjee, D. Madgwick, V. Akoyev, D.J. Takemoto. Biochemistry, Kansas State University, Manhattan, KS.

Purpose: Vertebrate lens primarily contains three connexin isoforms; connexin 43(Cx43), connexin 46(Cx46) and connexin 50(Cx50). The outer layer of epithelial cells in lens mainly contain Cx43 and Cx50 whereas the inner layer of differentiating fiber cells and mature fiber cells degrade Cx43 and preferentially contain Cx43 and Cx50. The purpose of this study is to investigate if overexpression of Cx46 mediates the degradation of Cx43 by the proteasomal pathway in lens.

Methods: The rat full length Cx46 cDNA was cloned into PEFGP-N3 vector and rabbit lens NN1003A epithelial cells were stably or transiently transfected with the construct. The level of Cx43 protein was determined by western blot and the level of Cx43 mRNA was determined by reverse transcription (RT)-PCR in transfected cells. The cells overexpressing Cx46 protein were treated with protease inhibitor cocktail, 100 uM of ALLN (N-Acetyl-Leu-Leu-Nle-CHO) + 10 uM of clostabilactacin ± Lactone to assay effects on Cx43 degradation. The increased ubiquitination of Cx43 in NN1003A cells overexpressing Cx46-GFP protein was investigated by immunoprecipitation studies where Cx43 was pulled down from whole cell lysate, by anti-Cx43 antibody followed by immunoblot with anti-ubiquitin antibody.

Results: Overexpression of Cx46 protein caused a decrease in endogenous Cx43 protein level both in stably or transiently transfected rabbit lens NN1003A epithelial cells. In transiently transfected cells the level of Cx43 protein was reduced at 36 h and 48 h after Cx46-GFP construct transfection. Cx43 mRNA level was not altered indicating reduction in Cx43 protein level is not due to inhibition of transcription. Treatment with protease inhibitor ALLN showed no change in Cx43 protein level even in NN1003A cells overexpressing Cx46 protein. Immunoprecipitation study showed increase in ubiquitin conjugation of Cx43 in cells that overexpress Cx46 as compared to control.

Conclusions: Our study demonstrates that overexpression of Cx46, initiates proteasome-dependent degradation of another gap junction protein, Cx43, in rabbit lens NN1003A epithelial cells.

CR: L.K. Bossmann, None; D. Banerjee, None; D. Madgwick, None; V. Akoyev, None; D.J. Takemoto, None.
Support: NIH EY13421.
Organizing Section: LE

4608 - D678

Endothelin-1 Inhibits Na,K-ATPase Activity Through a Src Family Kinase Mediated Mechanism in Porcine Lens Epithelium

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Methods: Porcine lenses were incubated in bicarbonate buffered Krebs’ solution at 37°C equilibrated with 5% CO2 and 95% air for a period of 0 to 30 min. Epithelia were carefully peeled off, homogenized in RIPA buffer and examined by western blot. Na,K-ATPase function in the intact lens was measured using ouabain-sensitive potassium (42K) uptake and by measurement of ouabain-sensitive ATP hydrolysis. Results: ET-1 caused a significant reduction in Na,K-ATPase activity as determined by direct measurement of ATP hydrolysis in intact lenses. ET-1 also caused a significant reduction in ouabain-sensitive potassium (42K) uptake. Both responses were abolished when lenses were pretreated with 10 μM PP2, a selective inhibitor of Src family kinases. Significant SKF phosphorylation in ET-1 treated lenses was determined as a ~61 kDa band recognized by the active loop Tyr-specific antibody. Furthermore, the phosphoryrosine band density was significantly reduced when an antibody against the C-terminal inhibitory Tyr-specific antibody was used. ET-1 caused a significant ERK1/2 phosphorylation, which also was abolished by PP2. Conclusions: The data point to an ET-1 mediated SKF activation which is associated with an inhibition of Na,K-ATPase pump activity. SKF activation precedes ERK1/2 activation in porcine lens. It remains to be determined whether SKF-mediated ERK1/2 activation plays a role in modulating Na,K-ATPase activity.


CR: A. Mandal, None; M. Shahidullah, None; C. Beimgraben, None; N.A. Delamere, None.

Support: NIH Grant EY009532

4610 - D680

The Beaded Filament Contributes Significantly to the Material Properties of the Lens

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Purpose: For normal function, the ocular lens must achieve and maintain optical clarity, as well as the exact geometry required to focus light, within narrow tolerances, on the retina. Additionally, in order to effect accommodation, the lens must be capable of shape change, followed by a return to its starting geometry. The molecular basis for the elastic properties that permit accommodation have not been identified. We hypothesized that the lens fiber cell-specific intermediate filament system (Beaded Filaments, or BF) conferred in whole, or in part, to the elastic properties of the lens.

Methods: Targeted genomic deletion of genes for both beaded filament proteins were generated previously. Electrophysiologic measurements were made from both wild-type and knockout mice. Filaments, or BF, conferred in whole, or in part, to the elastic properties that permit accommodation have not been identified. We hypothesized that the lens fiber cell-specific intermediate filament system (Beaded Filaments, or BF) conferred in whole, or in part, to the elastic properties of the murine lens and suggest that BFs are likely to be more important in humans and other species that rely more heavily on changes in material properties of the lens. knockout animal do not differ from those of the wild type, eliminating cell death as explaining this through the influence of physical stresses inside the lens. knockout analysis was carried out using a custom-designed program. Sizes of disordered cell patches and their location with reference to the epithelium were measured in ImageJ. To investigate packing order during the transition from epithelial to fiber cells, living lenses were stained with Hoechst for whole-mount confocal microscopy, and image stacks of epithelial and differentiating fiber cell nuclei at the equator were analyzed using a custom-designed program. Sizes of disordered cell patches and their location with reference to the epithelium were measured in ImageJ. To investigate packing order during the transition from epithelial to fiber cells, living lenses were stained with Hoechst for whole-mount confocal microscopy, and image stacks of epithelial and differentiating fiber cell nuclei at the equator were reconstructed in Volocity.

Results: Nearest neighbor analysis of the lens cortices shows patches of disordered fiber cell packing. Disruption of hexagonal packing does not arise during its establishment when the epithelial cells line up and differentiate to form fiber cells, but rather during fiber cell maturation as the cells move inwards to the lens cortex. High disorder in Tmod1/-/- lenses compared to Tmod1+/- lenses is caused by larger areas of individual patches of disordered fiber cells. However, the incidence of disordered patches in the lens cortex in the presence and absence of Tmod1 is similar. We present a possible mechanism explaining this through the influence of physical stresses inside the lens.

Conclusion: Tmod1 stabilization of the spectrin-based membrane skeleton is necessary to maintain hexagonal packing of fiber cells under the influence of physical stress.

CR: V.M. Fowler, None; M. Dressler, None; R.B. Nowak, None.

Support: NIH Grant EY019012 and NIH Grant AG032806

4611 - D681

Tropomodulin1 is Dispensable for Establishment But Not Maintenance of Fiber Cell Packing Geometry as Cells Mature in the Lens Cortex

V.M. Fowler, M. Dressler, R.B. Nowak. Cell Biology CB163, Scripps Research Institute, La Jolla, CA.

Purpose: Tropomodulin 1 (Tmod1) is a tropomyosin and actin binding protein that stabilizes the spectrin membrane skeleton in lens fiber cells and controls their hexagonal packing geometry (Nowak et al., 2009). To investigate the origins of disrupted hexagonal packing observed in lens fiber cells of a Tmod1 knockout mouse we compared packing geometries of fiber cells at different stages of maturation.

Methods: To quantify fiber cell packing order in the lens cortex, one-month-old mouse lenses were fixed, cryosectioned at the equator and stained with fluorescent- tagged antibody to outline fiber cell membranes for confocal microscopy. Nearest neighbor analysis was carried out using a custom-designed program. Sizes of disordered cell patches and their location with reference to the epithelium were measured in ImageJ. To investigate packing order during the transition from epithelial to fiber cells, living lenses were stained with Hoechst for whole-mount confocal microscopy, and image stacks of epithelial and differentiating fiber cell nuclei at the equator were reconstructed in Volocity.

Results: The data point to an ET-1 mediated SKF activation which is associated with an inhibition of Na,K-ATPase pump activity. SKF activation precedes ERK1/2 activation in porcine lens. It remains to be determined whether SKF-mediated ERK1/2 activation plays a role in modulating Na,K-ATPase activity.


CR: A. Mandal, None; M. Cooper, None; Y. Sun, None; R. Zhou, None.

Support: NIH Grant EY019012 and NIH Grant AG032806

4609 - D679

Temporal and Spatial Progression of Cataracts in the Ephrin-A5 Mutant Lens

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Purpose: The crystalline lens requires careful co-ordination of multiple signaling molecules to ensure proper development and maintain transparency. Disruptions in the control of these factors may result in disturbances leading to cataracts. Recently, we and several other studies have shown that the Eph family of receptor tyrosine kinase EphA2 and its ligand ephrin-A5 play an important role in lens development and function. Mutations in both the receptor and the ligand result in cataract formation. To elucidate the cellular mechanisms underlying the development of cataracts, we have identified the timing and location of the lens deficits in ephrin-A5-null mice.

Methods: Wild-type and ephrin-A5-null eyes at various ages were fixed, sectioned, and examined using H&E staining. Protein expression and localization were determined using immunohistochemistry and western blot analysis.

Results: Deficiencies in ephrin-A5 null lenses appear as early as P6, with >26% of the mutants developing large intercellular vacuoles in the fiber cell layer around the bow region. By P21, over 70% of ephrin-A5 mutants have developed large vacuoles between the fiber cells. The incidence rate increases to >86% by P180. Gross morphology of fibers during prenatal development appears normal. In contrast, no lens deformities are observed in the wild-type controls. Expression levels of several lens markers, including the crystallins and MIP-26, are also normal in ephrin-A5-null lenses.

Conclusion: These findings indicate that ephrin-A5 activity is indispensable in postnatal lens development. While the absence of ephrin-A5 does not impair initial lens formation, the regulation of fiber cell-cell interaction requires ephrin-A5 activity for maintaining proper lens structure.

CR: A.I. Soni, None; M. Cooper, None; Y. Sun, None; R. Zhou, None.

Support: NIH Grant EY019012 and NIH Grant AG032806

4608 - 4611

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4608-4611
4612 - D682
Solubility Properties of N-Terminal Domain, Core Domain, and C-Terminal Extension of Human αA- and βB-Crystallins

C.O. Asomugha, R. Gupta, O.P. Srivastava. Vision Sciences, University of Alabama at Birmingham, Birmingham, AL.

Purpose: To determine the solubility properties of the N-terminal domain, core domain and C-terminal extension of human αA- and βB-crystallins and to correlate to their biophysical properties and chaperone activity.

Methods: Wild type (WT) αA- and βB-crystallins, previously cloned in pET 100D TOPO vector, were used as templates to generate different constructs encoding specific regions (N-terminal domain [named as NT], core domain [named as CD], and C-terminal extension, [named as CT]). The specific regions amplified by PCR using plasmid DNA from WT αA and WT βB were: αA NT (residues 1-63), αA CD (residues 64-142), αA CT (residues 143-173), βB NT (residues 1-66), βB CD (residues 67-146), and βB CT (residues 147-175). Resultant blunt end PCR products were ligated to pET100 Directional TOPO vector. DNA sequencing was carried out to confirm the desired constructs. Plasmids were transformed into the expression cell line BL-21 Star (DE3), and expression and solubility of the proteins were confirmed by SDS-PAGE and by Western blot analysis using monoclonal antibodies against a 6x His tag.

Results: A total of six constructs were generated: αA NT, αA CD, αA CT, βB NT, βB CD, and βB CT. DNA sequencing confirmed all but one of the desired constructs of αA-crystallin and all constructs of βB-crystallin. Analysis of the expressed proteins by SDS-PAGE and Western blot methods showed that αA CD and βB CD were present in both the soluble and insoluble fractions. However, αA CD was present mostly in the insoluble fraction, unlike βB CD which was present mostly in the soluble fraction.

Conclusions: The temperature of solubility property of different regions of αA- and βB-crystallins is important for understanding their relative importance to the chaperone function of these crystallins. Based on the above results, the core region of αA-crystallin is relatively more insoluble than the core region of βB-crystallin. This property might affect the chaperone activity of both αA- and βB-crystallins.

CR: O.P. Srivastava, None; R. Gupta, None; O.P. Srivastava, None.
Support: EY06400

4614 - D684
Comparison of Thermally Adapted Fish αA-Crystallins Identifies an Amino Acid Substitution Associated With Chaperone-like Activity and Thermal Stability

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1Biology, Ashland University, Ashland, OH; 2Zoology, Miami University, Oxford, OH; 3OGVF/NEI, National Institutes of Health, Bethesda, MD.

Purpose: To use bony fish αA-crystallins as a model group to identify amino acid substitutions affecting thermal stability and chaperone-like activity and gain insights into small heat shock protein evolution.

Methods: Purified recombinant αA-crystallins from six fish species were previously cloned and used to identify correlations between physiological temperature, protein hydrophobicity, chaperone activity and thermal stability. The TreeSAP algorithm identified αA-crystallin regions undergoing natural selection for changes in hydrophobicity. Homology modeling using 3D-PSSM and PHYRE servers identified hydrophobicity, chaperone activity and thermal stability. The TreeSAPP algorithm identified amino acid substitutions that differed between species.

Results: Analysis of molecular adaptation and homology modeling of six bony fish αA-crystallins identified several residues differing in hydrophobicity that were predicted to affect thermal stability and chaperone-like activity. The single substitution Val27 to leucine in zebrafish αA-crystallin decreased thermal stability and increased chaperone-like activity compared to the wild type zebrafish protein. A second engineered mutation (C144S) did not alter thermal stability or chaperone-like activity at temperatures between 20-35°C, but showed minor elevated protein binding activity at 40°C. Molecular modeling of the zebrafish αA-crystallin domain suggests that the V27T mutation affects the structural stability of β-sheets. The C144S mutation is exposed at the domain surface and is expected to have a lesser effect on αA-crystallin stability.

Conclusions: These data indicate that change in a single amino acid residue can partially account for the thermal adaptation of vertebrate αA-crystallins, and that differences in hydrophobicity may underlie the functional effect of this variation. Further, this study validates a comparative evolutionary approach to analyzing structure/function relationships in small heat shock proteins, a protein family involved in numerous human diseases.

CR: J.M. Skiba, None; A.F. Drossman, None; A.J. Kiss, None; J.F. Hejtmanek, None; Y. Sergeev, None; M. Posner, None.
Support: NIH Grant EY013553

4615 - D685
Developmental Expression of βA3/1-Crystallin in the Rat Eye: From Structure to Function

G. Parthasarathy, C. Zhang, C. Bratyon, J.S. Zigler, J.S. Zigler, Jr., D. Sinha. 1Ophthalmology, Wilmer Eye Institute, Baltimore, MD; Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, Baltimore, MD.

Purpose: βA3/1-crystallin genes play a predominant role in maintaining the structure of the lens and may also be expressed in non-lens eye tissues. In previous studies, we reported a spontaneous mutation in the βA3/1-crystallin gene, Nuc1, with a novel eye phenotype. The current study was undertaken to analyze the expression of this gene during eye development in both wildtype and Nuc1 rats and to delineate its role in eye development.

Methods: Expression of βA3/1-crystallin was analyzed by in situ hybridization (ISH) and immunohistochemistry (IHC) during embryonic and post-natal development of wildtype and Nuc1 rats. For ISH, an antibody was generated to a peptide sequence common to both βA3 and βA1-polypeptides, and for IHC full-length anti-sense and sense probes were generated by standard protocols.

Results: βA3/1-crystallin was differentially expressed in different tissues of the eye with predominant expression in the lens. The lens expression was induced as early as E12.5 in the lens vesicle. Expression was primarily in the lens fibers and this pattern was seen throughout embryonic development and in the mature lens. Interestingly, expression in the retina is induced only in the first few days of post-natal development with no expression during embryonic stages. At 2 weeks post-natal, robust expression of the mRNA was seen predominantly in the ganglion cell layer and in the inner nuclear layer. In comparison to wildtype lens, Nuc1 lenses showed various developmental abnormalities with changes in the expression pattern for βA3/1-crystallin.

Conclusions: Expression of βA3/1-crystallin is controlled differentially in various eye tissues with lens being the most predominantly expressed tissue. Our studies provide novel evidence that βA3/1-crystallin likely plays a pivotal role in normal development and function of the eye, particularly in the remodeling process.

CR: G. Parthasarathy, None; C. Zhang, None; C. Bratyon, None; J.S. Zigler, Jr., None; D. Sinha, None.
Support: NIH Grants EY08365, EY019357 and EY019357-2S1, Research to Prevent Blindness and Helena Rubinstein Foundation (all to DS)

Wednesday, May 5, 1:45 PM - 3:30 PM Hall B/C Poster Session Program Number/Board # Range: 4600 - 4619 / D670 - D689

457. Proteins and Lipid Bilayers: Membrane Proteins, Cytoskeleton, Crystallins, Lipids

4612-4615
Characterization of a Transgenic Mouse Model Expressing Human Deamidated αA-Crystallin

R. Gupta, C. Asomugha, O.P. Srivastava. Vision Sciences, University of Alabama at Birmingham, Birmingham, AL.

Purpose: The purpose of the study was to characterize biochemical and morphological changes in transgenic mice lenses that were expressing deamidated αA-crystallin.

Methods: Transgenic mice expressing deamidated Asn at 101 residue in αA-crystallin were generated using mouse αA-crystallin promoter. Wild type (WT) αA-crystallin encoding amino terminal His-tagged cDNA was used to differentiate transgene expression from endogenous mouse αA-crystallin. Deamidation of Asn to Asp was introduced using a QuickChange site-directed mutagenesis kit (Stratagene). A transgenic mouse expressing WT αA-crystallin was used as a control (a gift from Dr. Mark Petrasek, University of Colorado, Denver). Biochemical properties of crystallins from both deamidated and WT mouse lenses were studied using gel filtration, 2D-DIGE gel electrophoresis, and immunohistochemical staining using a monoclonal antibody to the His-tag region (Novagen). Gross lens and fiber cell morphology were visualized with a digital camera attached to a stereo microscope and with Zeiss Axio Plan 2 imaging system, respectively.

Results: RT-PCR confirmed the transcription of transgene (NIID-mutant) and western blot/immunohistochemical staining confirmed the expression of the transgene in all independent founder lines compared to non-transgenic animals. Deamidated littermates showed relatively slow growth and slight decrease in lens weight compared to WT protein. The water soluble (WS)-protein content decreased with concomitant increase in water insoluble (WI)-proteins in deamidated lenses compared to WT lenses. Following size-exclusion chromatography of WS- proteins, the deamidated lenses showed a relatively decreased amounts of α- and β- crystallins compared to WT lenses. Relative to WT, the deamidated animals showed altered packing of fiber cells with aging. Further, a membrane protein, AQP0 was detected in higher levels in WI fraction of deamidated lens compared to WT lenses.

Conclusions: Expression of mutant NIID αA-crystallin results in higher level of WI-protein and altered packing of fiber cells in the transgenic mice lenses compared to WT mice.

CR: R. Gupta, None; C. Asomugha, None; O.P. Srivastava, None.
Support: EY08400

Novel Nonenzymatic Modifications of Lens Crystallins by Vitamin C Target Arginine Residues

I. Nemet, X. Fan, V.M. Monnier. Pathology, Case Western Reserve University, Cleveland, OH.

Purpose: Lens crystallins are prone to chemical modifications that affect chaperone function and favor toward aggregation and cataractogenesis. Vitamin C degradation products participate significantly in these chemical pathways in the lens. However, the products described so far can be formed from sugars other than Vitamin C and therefore do not reflect the specific role of Vitamin C in the modification. Here we characterized the structure of novel crystallin ascorbylation adducts and crosslinks with target amino acids and lens crystallins in vitro and from mice expressing the human vitamin C transporter 2 (hSVCT2) in the lens.

Methods: We protected amino acids, lysine and arginine were incubated with dehydroascorbic acid (DHA) under metal free conditions. Major incubation products were isolated by RP-HPLC and identified by NMR and mass spectrometry. These products were measured by LC-MSMS in bovine lens crystallins incubated with ascorbic acid or DHA (5 and 10 mM) for 7 days or crystallins from hSVCT2 mouse lens.

Results: From the Lys-Arg-DHA incubation two previously described crosslinks were isolated Lys-Arg-3-deoxyerythrosone (I) and Lys-Arg-erythrosone (II). In the arginine-DHA model systems three novel hydroimidazolone products derived from xylosone (3), erythrosone (4) and 3-deoxyerythrosone (5) were identified. In crystallins incubated in vitro with 5 mM ascorbic acid or DHA crosslink 3 reached the levels of 2.0 and 41.6 pmol/mg of protein, while crosslink 2 reached the levels of 66 and 664 pmol/mg of protein, respectively. Level of hydroimidazolone 3 was 10.9 pmol/mg of protein in crystallins incubated with 10 mM DHA. Surprisingly, only the hydroimidazolone 3 was identified in levels of 226 pmol/mg protein from 3 months old hSVCT2 mouse lens, but crosslinks 1 and 2 were not detected.

Conclusion: Novel products of protein modifications by Vitamin C which target arginine in crystallins were characterized and identified in vitro and in vivo systems. They could destabilize arginine residues and contribute toward impaired chaperone function during aging.

CR: I. Nemet, None; X. Fan, None; V.M. Monnier, None.
Support: EY07099, VSCR P30EY11373, and JDRF fellowship to IN

Alpha A-Crystallin Inhibits Apoptosis by Inactivating PTEN

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Purpose: Alpha crystallin functions as a molecular chaperone and as an anti-apoptotic protein using two mutant proteins, R21A and R49A, which exhibit higher and lower chaperone activity relative to the wild type protein, respectively.

Methods: We generated Chinese Hamster Ovary (CHO) cell lines that stably overexpress either the wild type (WT) or R21A or R49A mutant protein. The same three proteins were transiently overexpressed in HeLa cells. Apoptosis was induced by either staurosporine or etoposide or doxorubicin. Phosphorylation of Akt, PDK1 and PDK3 and PTEN was assessed by western blotting. PI3K activity was measured using a commercial kit. P3K/ninase downregulation was achieved by expression of a dominant negative mutant.

Results: R21A cells showed enhanced protection and R49A cells showed reduced protection against apoptotic agents, relative WT cells. This effect was due to higher PI3K activity in R21A cells, although the PI3K activity were similar in all cell lines. Akt phosphorylation was higher in R21A cells when compared to either R49A or WT cells. Downregulation of PI3K was by a dominant negative mutant or inhibition by LY294002 augmented the enhanced ability of R21A to phosphorylate Akt, suggesting that αA-crystallin inhibits apoptosis through activation of PI3K. PI3K and PTEN phosphorylation were increased in R21A cells relative to WT cells.

Conclusion: Our data reveal that αA-crystallin inhibits apoptosis by enhancing PI3K activity and inactivating PTEN and that the anti-apoptotic function is directly related to its chaperone activity.

CR: N. Pasupuleti, None; S. Matsuyama, None; O. Voss, None; A. Dosef, None; K. Song, None; D. Danielpour, None; R.H. Nagaraj, None.
Support: RO1EY-061219, RO1EY-09912, RO1HL75040-01, RO1AG01903, P30EY11373, RFB and OLERF

Alpha A-Crystallin Mutants Causing Congenital Cataracts

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Purpose: Pediatric cataract of the congenital type is the most common cause of childhood blindness. Mutations in 22 different genes have been identified to be associated with congenital cataracts and, among them, 8 mutants belong to αA-crystallin. To explain how each mutation in αA-crystallin leads to the development of cataract, structural, functional, hydrodynamic and interactive properties of the mutants were studied.

Methods: αA-crystallin (αA-wt) and its site-directed mutants, namely, R12C, R21L, R21W, R49C, R54C, R116C and R116H were generated by Quick-Change site-directed mutagenesis kit, expressed in E.coli BL21 (DE3) PLYS5 cells, and purified by size-exclusion chromatography. Molar mass, hydrodynamic radius (Rh), and polydispersity index (PDI) were assessed by dynamic light scattering measurements, chaperone activity was measured with ADH and β-crystallin as target proteins. Conformational studies included CD measurements and TNS binding and the interaction of the mutants with ββ-crystallin was investigated by the interactive CD approach.

Results: The average molecular mass and mass across the peak for αA-wt and the mutants showed substantial increase in R16C and R16H, moderate increase in R12C, slight increase in R21W and R54C and no increase in R21L and R49C. PDI and Rh values were significantly increased only in R16C and R16H. Relative chaperone activity was decreased in R12C, R16C and R16H, but remained unchanged in all other mutants. Changes in the secondary structure in the form of an increase in the o-helix content were observed in R12W, R21L, R16C and R16H. Tertiary structural changes were evident in R12W, R54C, R16C and R16H. Maximum level of interaction with ββ-wt was shown by R16H, R21W, R16C and R21L, in that order; the same mutants showed the highest level of secondary structure change.

Conclusions: Although a specific change in αA-crystallin behavior is common to all αA mutants, the mutants was not observed, each mutant, however, showed one or more perturbation as probable cause of cataract. The highly conserved residue R116 which exists in the “α-crystallin” domain remains as a “hot spot” showing substantial changes in its oligomeric structure after its mutation to Cys or His. Comparison of R116C and R116H, strongly suggests that mutation of R to C is not essential for showing such drastic changes in the αA-crystallin structure.

CR: R.A. Kore, None; P. Santhoshkumar, None; K. Sharma, None; E.C. Abraham, None.
Support: NIH/EY11352
466 - Genetics

467 - 4:15PM
An Animal Model to Study the Dynamic Regulation of Glutathione in the Lens: The Lenticular Gamma Glutamate-Cysteine Ligase Conditional Knockout Mouse (Preliminary Studies)

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Purpose: Glutathione (GSH) is the most abundant small molecular thiol compound found in aerobic eukaryotic organisms. It fulfills numerous cellular functions, including protection against oxidative stress, control of cellular redox potential and detoxification of xenobiotics. GSH is also a principle antioxidant keeping lens crystallin damage in check. Lens is now recognized as a very high level of GSH by mammalian milligram concentrations. Biochemical synthesis of GSH occurs through the sequential action of two ATP-dependent enzymes: γ-glutamate cysteine ligase (GCL) and glutathione synthetase (GS). The systematic knockout of Gcl leads to embryonic lethality in mammals and is not used for lentil studies.

Methods: The Cre-Lox site-specific recombination system was used by flank γ-glutamate cysteine ligase catalytic subunit (Gclc) exons 2 with two Loxp sites developed by European Conditional Mouse Mutagenesis Program. The Flp-Frt site-specific recombination was used to flank Neocarboxin cassette, and eventually was removed via cross-breeding with Flp transgenic mouse. LoxP-flanked Gclc mice was cross-bred with MRL-10 lens Cre transgenic mouse to create the lens conditional Gclc knockout mouse. The breeding was selected by regular PCR with specific primers through tail genomic DNA extraction. The Gclc gene expression and protein level were determined by real time PCR and Western blot respectively.

Results: Heterozygous knockout mouse showed 30-45% drop in mRNA level of Gclc and about 40-50% drop in lens compared to wild type mice. Surprisingly, the GSH level only dropped around 10% compared to wild type lenses. These results indicated that the dynamic regulation of GSH in lens occurs both by de novo synthesis and extracellular uptake via GSH transporter which has been reported before. The compensation from GSH transport indicates presence of a high affinity transporter in lens epithelial cells, because the GSH concentration in aqueous is in micromolar, while in lens in millimolar level.

Conclusions: A conditional ko mouse for GSH synthesis in the lens has been created as an initial step for the study of impaired GSH levels in human cataract. The heterozygous mouse shows only a modest drop in GSH that is compensated by lenticulat uptake via a yet unknown high affinity transporter.

CR: X. Fan, None; S. Hao, None; X. Liu, None; M.L. Robinson, None; V.M. Monnier, None.

Support: EY 07099 and the VSRC grant P30EY-11373

470 - 4:00PM
Role of ADAMTS4 Mutations in FBNI Mutation Negative Ectopia Lentis Patients

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Purpose: Although clinically homogeneous, ectopia lentis (EL) is genetically heterogeneous with both autosomal-dominant (MIM 129600) and autosomal-recessive (MIM 225100) forms. The dominant disorder can be caused by mutations in FBNI, at the milder end of the type-1 fibrillinopathy spectrum. Recently in a consanguineous Jordanian family, recessive EL was mapped to locus 1q21 containing the ADAMTS4 gene and a nonsense mutation found in exon 11 (c.1785T>G, p.Y595X) of ADAMTS4.

Methods: In this study, 12 consecutive Caucasian U.K. probands with EL and demonstrating no, or very mild, heart involvement on echocardiogram were included. Probands did not fulfill the Ghent criteria for Marfan syndrome and were previously found mutation-negative for FBNI. Mutation screening in ADAMTS4 by direct sequencing of all exons including their intron/exon boundaries was performed.

Results: Homozygous or compound heterozygous mutations were identified in 6/12 (50%) probands. Mutation data are summarised in table 1. Where available, familial screening of these families confirmed the mutation co-segregated with the EL phenotype. None of the ADAMTS4 mutations described here were identified in controls or normal control chromosomes.

Conclusions: This study is the first confirmation that homozygous mutations in ADAMTS4 are associated with autosomal recessive EL. The first compound heterozygous mutations are described. The identification of a causative mutation in ADAMTS4 may allow exclusion of Marfan syndrome in these families and guide clinical management, of particular relevance in young children affected by EL.

CR: A.H. Child, None; J.A. Aragon-Martín, None; D. Ahnoud, None; D. Charteris, None; A. Saggar, None; G. Arno, None.

Support: Marfan Trust UK, Moorfields Eye Hospital, UK

471 - 4:15PM
Characterization, Distribution and Putative Function of ADAMTS4, an Extracellular Protein Mutated in Recessive Ectopia Lentis

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Purpose: ADAMTS4 mutations were recently identified in AR isolated ectopia lentis. To date, 9 pathogenic mutations were identified in ADAMTS4. We characterized the expression and function of ADAMTS4.

Methods: ADAMTS4 cDNA was cloned in a pcDNA3.1 vector in-frame with a C-terminal Myc+6XHis tag and transfected into HEK293F cells to characterize the recombinant protein. ADAMTS4 polycistronic Ab was characterized by immunoblotting of medium and the cell lysate, and comparison with anti-Myc Ab. Extracted protein from different structures in healthy human eye tissue was analyzed by western blotting with ADAMTS4 Ab, and immunoperoxidase staining of sections of normal and Marfan syndrome human eyes were performed to determine the ocular distribution of ADAMTS4. To check whether ADAMTS4 influenced FBNI deposition in extracellular matrix, we used a functional assay in which fetal bovine nuchal ligament cells (FBNLCs) were incubated with medium containing ADAMTS4 or a control medium, and FBNI immunofluorescence was measured.

Results: ADAMTS4 DNA sequence predicts a core protein of 140 kDa with substantial N-glycosylation. Western blotting of recombinant protein with ADAMTS4 Ab showed a major band of 150 kDa in ADAMTS4-transfected but not vector-transfected cells, similar to recombinant with anti-Myc Ab. By tissues western blots, ADAMTS4 protein was detected in the uveal tract, lens capsule, zonule fibers, cornea, and retina. Immunoperoxidase staining of one healthy eye and one Marfan syndrome eye showed primarily a fibrillar, extracellular staining in most of the eye, and was not restricted to the zonular apparatus. In the presence of ADAMTS4-transfected medium, FBNLCs showed enhanced FBNI deposition compared with the control.

Conclusions: ADAMTS4 is a secreted glycoprotein that is widely distributed in the human body, including regions where the zonular apparatus is assembled. Our in vitro data demonstrating enhanced FBNI-1 deposition in the presence of ADAMTS4 suggest a role in the interaction of these proteins in the assembly of the zonule.

CR: L.A. Gabriel, None; J.G. Hollifield, None; E.I. Troubski, None; A.K. Majors, None; S.S. Apt, None.

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4774 - 5:00PM  
Mammalian Lens Placode Invagination Requires Cdc42, RhoA and Rac1  
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1The Visual Systems Group, Division of Pediatric Ophthalmology,  
2Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH;  
3Dept. of Ophthalmology, University of Cincinnati, Cincinnati, OH;  
4School of Medicine Dept., IFOH Foundation, Institute FIRC of Molecular Oncology, University of Milan, San Paolo, Via Adamello,  
5University of California San Francisco, Department of Ophthalmology, San Francisco, CA;  
6Dept. of Chemistry and Physics, Lamar University, Beaumont, TX.  
Purpose: The formation of the mammalian lens pit provides an excellent model to study the morphogenetic pathways involved in epithelial invagination. In this study, we attempt to address the importance of three chief small Rho GTPases, Cdc42, RhoA and Rac1, in this process.  
Methods: Three conditional mouse mutants of Cdc42, RhoA and Rac1 were crossed with the floxed mouse line to generate corresponding deleted alleles only in the induced lens ectoderm. Analysis, including immunofluorescent-staining and quantification, was conducted at specific stages of lens placode invagination.  
Results: We show that F-actin-rich filopodia link adjacent presumptive lens and retina. The filopodia, most of which originate in the presumptive lens, form at E9.5 when presumptive lens and retina first come into close contact, and have retracted by E11.5 when invagination is complete. Formation of filopodia is dependent on Cdc42 and its effector BRS53 (Baiap2). Loss of filopodia results in reduced lens pit invagination. Pharmacological manipulation of the actin-mysin contraction pathway showed that the filopodia can respond rapidly in length to change the inter-epithelial distance. Next, we show that there are apical actomyosin complexes in the lens placode that respond to pharmacological manipulation, thereby reducing invagination. We observe that activated myosin and F-actin is reduced in RhoA mutant lens pits, therefore producing shallower pits. In contrast, early invagination of placentas occurs in the Rac1 mutants compared to wild-types. Interestingly, the lens pits are thinner in the Rac1 mutants, but noticeably thicker for the RhoA mutants.  
Conclusions: Collectively, our results suggest that basal lens filopodia provide a physical tether that coordinates invagination and correct positioning of the lens in the early eye. The future work is to regulate the apical actomyosin complexes that drive invagination, and Rac1 is responsible for migration of the lens placode towards the presumptive retina.  
CR: B.K. Chauhan, None; A. DiSanza, None; S.-Y. Choi, None; M. Lou, None; H.E. Beggs, None; G. Scita, None; Y. Zheng, None; R.A. Lang, None.  
Support: NIH Grants EY15766, EY16241, EY17848, CA131270 to R.A.L., EY0117379 to H.E.B., HL085362 to Y.Z. and the Abrahamson Pediatric Eye Institute Endowment to R.A.L.
Organizing Section: LE

518. Cataract Surgery

Support: CR:

A study may serve as a pilot for a larger study to evaluate and quantify the impact of an anesthetic intervention on vision-related quality of life in cancer patients. This study is summarized.

Compared to pre-operative NEI-VFQ scores, cancer patients had a significant increase in vision-related quality of life (P=0.000074) after the surgery. Patients also had a statistically significant improvement in eight of the twelve subcategories in the NEI-VFQ.

Conclusions: Patients having cataract surgery experienced less intra- and postoperative anxiety if they heard no music or music they had selected themselves as opposed to music assigned by the operating room experience. Our study aims to determine the impact of music in the operating room and patient anxiety during cataract surgery.

CR: B.A. Karwoski, None; T.M. Kazam, None; J.M. Solomon, None. Support: None.

5384 - A400

Ischemic Diabetic Retinopathy Protects Against Nuclear Cataract After Vitrectomy

Y.-B. Shui, N.M. Holekamp, F. Bai; A. Almony, D.C. Beebe. Dept of Ophthal & Vis Sciences, Washington Univ Sch of Med, St Louis, MO; Barnes retina Institute, St Louis, MO.

Purpose: Patients with diabetes have significantly lower vitreous pO2, and are less likely to have cataract surgery after vitrectomy than non-diabetics. Based on the hypothesis that oxygen exposure to the lens is a major risk for nuclear cataract, we performed a prospective interventional cohort study to determine whether diabetes mellitus protects against nuclear cataract after vitrectomy.

Methods: Phakic diabetics and non-diabetics undergoing vitrectomy surgery for a variety of retinal conditions underwent Scheimpflug lens photography (NIDEK EAS-1000, Japan) in the examined and fellow eye before, and 6 and 12 months after surgery. Images were analyzed with the EAS-1000 analysis software. Nuclear opacity was quantified using average light scattering per pixel on the central line between the anterior surface of adult nucleus and the clear zone at the center of the lens.

Results: Of 55 patients included in the analysis, 24 had diabetes, 16 of which had surgery for complications of ischemic retinopathy. The remaining 8 diabetics did not have ischemic retinopathy. None of the patients with diabetic diabetic retinopathy had significantly less nuclear opacity than non-ischemic diabetic and non-diabetic subjects (p=0.001 in surgical eyes, p=0.002 in fellow eyes). After vitrectomy surgery, non-ischemic diabetic eyes and non-diabetic eyes developed nuclear cataract (P=0.05 compared to baseline at 6 and 12 months). However, eyes with ischemic diabetic retinopathy showed no significant progression of nuclear opacification and, therefore, had significantly less post-vitrectomy nuclear cataract at 6 (p=0.0001) and 12 months (p=0.0005) than non-diabetic and non-ischemic diabetic eyes. Adjusting for baseline opacity and age did not alter this result. The cataract surgery rate was 50% in the non-ischemic eyes and 16% in the ischemic eyes at the end of one year.

Conclusions: Ischemic diabetic retinopathy, but not diabetes alone, protected against nuclear opacity at baseline and the development of nuclear cataract following vitrectomy. These findings are consistent with the hypothesis that increased exposure to oxygen is responsible for nuclear cataract formation in humans.

CR: Y.-B. Shui, None; N.M. Holekamp, None; F. Bai, None; A. Almony, None; D.C. Beebe, None. Support: NIH Grant EY015863, unrestricted grant, NIH Vision Core Grant P30 EY08687.

5385 - A401

Ischemic Diabetic Retinopathy Protects Against Nuclear Cataract After Vitrectomy

Y.-B. Shui, N.M. Holekamp, F. Bai; A. Almony, D.C. Beebe. Dept of Ophthal & Vis Sciences, Washington Univ Sch of Med, St Louis, MO; Barnes retina Institute, St Louis, MO.

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Conclusions: Ischemic diabetic retinopathy, but not diabetes alone, protected against nuclear opacity at baseline and the development of nuclear cataract following vitrectomy. These findings are consistent with the hypothesis that increased exposure to oxygen is responsible for nuclear cataract formation in humans.

CR: Y.-B. Shui, None; N.M. Holekamp, None; F. Bai, None; A. Almony, None; D.C. Beebe, None. Support: NIH Grant EY015863, unrestricted grant, NIH Vision Core Grant P30 EY08687.

5386 - A402

Cataract Surgery in Cancer Patients: Its Impact on Quality of Life as Measured by NEI-VFQ

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Purpose: The measurement of quality of life has become an important topic in healthcare and in the allocation of limited healthcare resources. Improving the quality of life for those who may have a shortened life expectancy is often the goal of treating cancer patients. The purpose of this research is to quantify the change in vision-related quality of life in cancer patients as measured by the National Eye Institute Visual Functioning Questionnaire (NEI VFQ-25) who underwent cataract surgery at a tertiary cancer hospital.

Methods: An IRB-approved pilot study was performed in cancer patients undergoing cataract surgery from the MD Anderson Cancer Center. Patients who completed the NEI VFQ-25 before and 4 to 6 weeks after cataract surgery were evaluated. The responses of NEI-VFQ-25 were analyzed using the Wilcoxon matched pairs test.

Results: 34 patients were included in the analysis from a single surgeon’s practice. Compared to pre-operative NEI-VFQ scores, cancer patients had a significant increase in vision-related quality of life (P=0.000074) after the surgery. Patients also had a statistically significant improvement in eight of the twelve subcategories in the NEI-VFQ. Details of the patients’ demographics, cancer history, surgical outcomes are summarized.

Conclusions: Cataract surgery appears to impact significant improvement on the vision-related quality of life in cancer patients as measured by NEI-VFQ. The benefit of cataract surgery in the cancer patients has not been previously published. This study may serve as a pilot for a larger study to evaluate and quantify the impact of an elective surgical intervention on quality of life in the cancer population.

CR: T.L. Jackson, Jr., None; V. Arevalo, None; D. Roberts, None; S.K. Kim, None. Support: Charles H. Griffenberg Memorial Fund.

5387 - A403

Endophthalmitis Prophylaxis: A Single Center’s Experience and Critical Review of ESCRS Guidelines

T. Ness, W.Y. Kern, U. Frank, T. Reinhard. Univ Eye Hospital, Center for Infectious Diseases and Travel Medicine, Department of Environmental Health Sciences, University of Freiburg, Freiburg, Germany.

Purpose: To report the rate of endophthalmitis after cataract surgery in patients given preoperatively topical povidone-iodine and gentamicin-containing irrigation fluid as prophylaxis without intracameral cefuroxime or postoperative topical levofloxacin. The purpose of this research is to quantify the change in vision-related quality of life in cancer patients as measured by the National Eye Institute Visual Functioning Questionnaire (NEI VFQ-25) who underwent cataract surgery at a tertiary cancer hospital.

Methods: In a retrospective clinical study, we included patients undergoing cataract surgery within a 12-year period at a large single medical center. Cases of postoperative endophthalmitis were identified (rate, 0.6 per 1,000 operations, or 0.06%, 95% confidence interval, 0.03-0.09%). A causative micro-organism was detected in 81.3% (13/16) of the cases. Most organisms were gram-positive bacteria (11/13) with susceptibility to cefuroxime, aminoglycosides, and fluoroquinolones. The three gram-negative pathogens were susceptible to cefuroxime, aminoglycosides, and fluoroquinolones.

Conclusions: Using our regimen of topical povidone-iodine and gentamicin-irrigation fluid as prophylaxis without intracameral cefuroxime or postoperative topical levofloxacin, we suggest that the ESCRS guidelines should be substantially revised to allow alternative effective regimens for the prevention of postoperative infections following cataract surgery.

CR: T. Ness, None; W.Y. Kern, None; U. Frank, None; T. Reinhard, None. Support: None.
Cataract Surgery postoperative ocular complications from cataract surgery in the United States (US) Award Program, Veterans Affairs Medical Center, Providence, RI; Division of Ophthalmology, Department of Medicine, Warren Alpert Medical School of Brown University, Providence, RI; Ophthalmic & Public Health Sciences, Penn State College of Medicine, Hershey, PA.

**Purpose:** To investigate the prevalence and predictors of intraoperative and 90-day postoperative ocular complications from cataract surgery in the United States (US) Veterans Health Administration (VHA).

**Methods:** The VHA National Patient Care Database (NIPCD) was used to identify all patients who underwent outpatient extracapsular cataract surgery and had only one cataract surgery within 90 days of the index surgery between October 1, 2005 and September 30, 2007. The prevalence of demographic factors, preoperative systemic and ocular comorbidities, intraoperative complications, and 90-day postoperative complications was collected. Predictors of complications were identified by calculating adjusted odds ratios (OR) using logistical regression modeling.

**Results:** Of 53,786 veterans who underwent cataract surgery during the study period, 45,082 met inclusion criteria. The most prevalent preoperative systemic and ocular comorbidities included diabetes mellitus (40.6%), chronic obstructive pulmonary disease (21.2%), age-related macular degeneration (14.4%), and diabetes with ophthalmic manifestations (14.0%). The most prevalent ocular complications were posterior capsular tear and/or anterior vitrectomy intraoperatively (5.9%) and posterior capsular opacification postoperatively (4.2%). Significant predictors of complications included: black race (OR 1.38 [95% confidence interval, 1.28-1.50]), township (OR 1.10 [1.03,1.18]), married (1.26 [1.14,1.38]), diabetes with ophthalmic manifestations (1.33 [1.23,1.43]), traumatic cataract (1.80 [1.40,2.31]), previous surgical eye (1.29 [1.02,1.63]), and older age.

**Conclusions:** Selected demographic factors and ocular comorbidities were associated with greater risks of cataract surgery complications in the VHA. Further large-scale studies are warranted to investigate cataract surgery outcomes for other US patient populations.

**CR:** V.L. Tseng, None; P.B. Greenberg, None; W.-C. Wu, None; J. Liu, None; L. Jiang, None; C.K. Chen, None; E.U. Scott, None; P.D. Friedmann, None.

**Support:** Center on Systems, Outcomes, and Quality in Chronic Disease and Rehabilitation, Research Enhancement Award Program, Health Service Research and Development (HSR&D) Service Grant number REA08-263

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**Organizing Section: LE**

**5388 - A405** Histopathological Findings of the Anterior Lens Capsule in Vitrectomized Patients With Silicone Oil Tamponade

I.M. Zand-Hadas, E. Fernández-Muñoz, A.A. Lorenzo, A.A. Rodríguez-Reyes, I.U. Scott

**Ophthalmology, Ophthalmic Pathology, Asociacion para Evitar la Ceguera, Mexico, Mexico.

**Purpose:** To describe the histopathological changes of the anterior lens capsule (ALC) in vitrectomized patients with silicone oil tamponade.

**Methods:** We included samples of ALC from consecutive eyes of patients with proliferative diabetic retinopathy and history of vitrectomy and silicone oil tamponade that underwent cataract surgery. The samples were formalin-fixed and paraffin embedded tissue. From each sample, 4 histological sections were obtained. All sections were stained with hematoxylin-eosin, periodic acid, and Sirius red dye and examined using light microscopy and polarized light. We evaluated capsular thickness (CT), epithelial features and the presence of different types of collagen. ALC of diabetic patients without prior vitrectomy were used as controls.

**Results:** We analyzed ten samples of ALC. Two of them were from vitrectomized patients and eight from non-vitrectomized patients. From the two vitrectomized samples, both had silicone oil as tamponade. CT was greater by more than 3μm in samples obtained from vitrectomized patients. Hyperplasia, fibrosis and inflammatory cells were found in one of them. Type 1 and 3 collagen was not found in vitrectomized samples.

**Conclusions:** A greater CT and the presence of capsule fibrosis may explain the empirical observation of a different capsular consistency during capsulorrhexis in vitrectomized patients. Contrary to our hypothesis, collagen types 1 and 3 were not found in the ALC.

**CR:** I.M. Zand-Hadas, None; E. Fernández-Muñoz, None; A.A. Lorenzo, None; A.A. Rodríguez-Reyes, None.

**Support:** None

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**5390 - A406** Is it Still a Challenge to Perform Cataract Surgery After Pars Plana Vitrectomy? Retrospective Study About 30 Cases


Ophthalmology, Regional Hospital Center Bessecours, Metz, France.

**Purpose:** The authors attempt to list the possible difficulties met during 2.2 mm microincision cataract surgery, on previously vitrectomized eyes. All the analyzed cases were of important hardness, classified in rank 3 or 4 of cataract evolution.

**Methods:** From October 08 to October 09, 30 previously vitrectomized eyes (indication of vitrectomy was vitreous haemorrhages secondary to proliferative retinopathy, with temporarily silicone oil tamponade), are operated by 2.2 mm coaxial microincision phacoemulsification, with the device Stellars Bausch & Lomb, with final foldable IOL implantation, by the same surgeon. The patient’s characteristics were analysed, duration of intervention, types of IOL, evolution of visual acuity, the difficulties faced and possible complications.

**Results:** The study is retrospective, involving 30 eyes of 22 men (73.33 %) and 8 women (26.66 %). The age at the time of surgery is about 6 months ranging from 1 to 12 months), the average age of the patients is about 63,57 years (ranging from 47 to 79 years), the average duration of intervention is about 11,05 minutes (ranging from 8 to 20mn), the average age of survive patients is 96.7 5 years after surgery, and to 96.4 10 years after surgery. Ten years after surgery, 46 patients (16%) had a decline in BCVA of more than 0.5 logMAR units, compared with postoperatively. Forty-six patients (16%) had a decline in BCVA of more than 0.3 logMAR units. The median VF-14 total score was 100 after surgery, declining to 96.7 5 years after surgery, and to 96.4 10 years after surgery. Ten years after surgery 49% of the patients had unchanged or better subjective visual function and 77% had less than 10 points in decline compared with postoperatively. Nine percent (31 of 335 patients) had a worsening of VF-14 total score of 40 points or more. In this group age-related macular degeneration (ARM) was the most common cause followed by bullous keratopathy. Only 18% of the patients in this cohort had an unoperated fellow eye 10 years after surgery.

**Conclusion:** Longitudinal follow-up of this older population confirms that subjective and objective visual function 10 years after cataract surgery remains stable in most surviving patients. Cataract surgery offers good long-term visual rehabilitation for cataract. Common morbidity, most commonly ARM but also bullous keratopathy, are the most important causes for deterioration of visual function.

**CR:** E. Monestam, None; L. Lundqvist, None.

**Support:** Grants by Västerbottens County Councils, Crown Princess Margaretas Committee for the blind and Swedish Medical Society, Stockholm, Sweden

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**5391 - A407** Longitudinal Visual Outcome 10-Years After Cataract Surgery


**Purpose:** This prospective, population-based cohort study reports the longitudinal visual functional outcome of cataract surgery on a long-term basis. Data collected pre- and postoperatively, as well as 5 and 10 years after surgery from the same patients is analyzed.

**Methods:** 810 participants underwent cataract surgery during a 1-year period. Before surgery, a few months after surgery, and 5 and 10 years after surgery, age and comorbidities were recorded, and BCVA was measured. At all occasions the patients also answered the same visual functional questionnaire (VF-14). Ten years after surgery 335 (85% of survivors) participated with the questionnaire of whom 289 (73% of survivors) were re-examined.

**Results:** Ten years after surgery 76% of the patients examined had a BCVA of 20/40 or better, and 65% had a BCVA of 20/30 or better, which is a decline from 88% and 84% after surgery, respectively. Regarding each operated eye, 50% had unchanged or better VF, and an additional 19% had only lost less than 0.1 logMAR units, compared with postoperatively. Forty-six patients (16%) had a decline in BCVA of more than 0.3 logMAR units. The median VF-14 total score was 100 after surgery, declining to 96.7 5 years after surgery, and to 96.4 10 years after surgery. Ten years after surgery 49% of the patients had unchanged or better subjective visual function and 77% had less than 10 points in decline compared with preoperatively. Nine percent (31 of 335 patients) had a worsening of VF-14 total score of 40 points or more. In this group age-related macular degeneration (ARM) was the most common cause followed by bullous keratopathy. Only 18% of the patients in this cohort had an unoperated fellow eye 10 years after surgery.

**Conclusion:** Longitudinal follow-up of this older population confirms that subjective and objective visual function 10 years after cataract surgery remains stable in most surviving patients. Cataract surgery offers good long-term visual rehabilitation for cataract. Common morbidity, most commonly ARM but also bullous keratopathy, are the most important causes for deterioration of visual function.

**CR:** E. Monestam, None; B. Lundqvist, None.

**Support:** None
Cataract Surgery

5392 - A409
Pre-Existing Ocular Conditions and Complication Risk in Cataract Surgery
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Purpose: Cataract operations are the most common elective procedure performed in the UK. Advances in surgical technique have greatly reduced the risk associated with such operations. However, complications do still occur and can cause long-term detriment to the patient. Previous studies have addressed complication rates but the relationship between specific pre-existing conditions and complication risk has not yet been fully elucidated. The purpose of this study was therefore to determine whether pre-existing ocular conditions increase the risk of mild, moderate or severe complications and to quantify any such relationships.

Methods: An electronic data set was prospectively compiled for cataract operations performed at King’s College Hospital from 2006-9. Information gathered included pre-existing ocular conditions and surgery outcome. Operations with no pre-existing conditions were classified as control. All others were grouped according to condition type. The frequencies of mild, moderate and severe complications in each group were compared with control using a chi-squared test. Relative risks (RR) were then calculated for each statistically significant result (p<0.05).

Results: 7702 control operations and 2071 operations with pre-existing ocular conditions were included. Total complication rates were 2.64% and 6.81% respectively. The risk of mild complications was significantly increased by pre-existing uveitis/ synchiae, pseudoxfoliation/phacodonesis, brunescent/white cataract, high myopia, diabetes, retinopathy or previous vitrectomy with RR of 3.69, 6.22, 7.46, 2.64, 3.12 and 9.54 respectively. The risk of moderate complications was significantly increased by corneal pathology, pseudoxfoliation/phacodonesis, brunescent/white cataract or reduced fundal view/retrovascular opacities with RR of 2.94, 8.92, 9.62 and 8.79 respectively. The risk of severe complications was significantly increased by brunescent/white cataract only with RR of 15.97.

Conclusions: We have shown that the risk of complications is affected by varying degrees by different pre-existing ocular conditions. The results of this study will therefore enable surgeons to provide more individualised counselling to patients before undergoing cataract surgery.

CR: K.L. Whitcroft; None; N. Patel; None; G. Larkin; None.
Support: None

5393 - A409
3D-Evaluation of Visoscoinative Ophthalmic Viscosurgical Devices (OVDs) During Cataract Surgery Using the Pentacam Scheimplug Camera
B. Lundgren1, M. Lundqvist2, S. Nilsson3; R & D, Abbott Medical Optics Inc., Upssala, Sweden; R & D, Abbott Medical Optics Inc, Upssala, Sweden.

Purpose: Phacoemulsification with use of an OVD is the preferred technique of modern cataract surgery. The OVD maintains the anterior chamber, protects the corneal endothelium and facilitates IOL implantation. By using viscosurgical OVDs, the benefits of both the cohesive (anterior chamber stabilization) and dispersive (corneal protection) features can be utilized. The purpose of this study was to objectively evaluate the performance of the viscosoaptive Healon5 OVD (AMO) with Microvisc Phaco (BD/ Bohor Biotech) and the viscous dispersive OVD, Discovisc (Alcon), during simulated cataract surgery.

Methods: Cataract surgery was simulated ex vivo in porcine eyes. OVDs were stained with fluorescein for detection purposes. Each step in the surgeries (before surgery, after OVD injection, after hydrodissection, and after phacoemulsification) was monitored by multiple Scheimplug photographs using the Pentacam HR (Oculus) and anterior chamber depths were measured. The tomography 3D-model was used to further visualize and evaluate the OVD performance. Three different phacoemulsification flow and vacuum settings (low, medium, and high) were used in order to cover the most clinically relevant settings.

Results: This Scheimplug camera study reveals superior properties of Healon5 OVD as the only OVD with consistent anterior chamber behavior throughout the study. Among the tested OVDs, Healon5 was the only OVD that stayed in close contact with the corneal endothelium during phacoemulsification. In approximately half of the surgical cases with Microvisc Phaco or Discovisc these OVDs were partly or completely detached from the corneal endothelium by the end of phacoemulsification. The 3D tomography visualized these findings further in an objective way.

Conclusions: Of the three different OVDs tested, only the Healon5 OVD showed true viscosoaptive behaviour, meaning consistent anterior chamber maintenance capacity and good retention during all phacoemulsification settings tested. Even though the other tested OVDs were still present in the anterior chamber, their loose positioning gives less control of endothelium protection. The Pentacam 3D tomography software tool can objectively visualize differences in OVD behavior during cataract surgery.

CR: B. Lundgren; Abbott Medical Optics, E; M. Lundqvist; Abbott Medical Optics, E; S. Nilsson; Abbott Medical Optics, E.
Support: None

5394 - A110
Clinical Outcomes Following Laser Cataract Surgery
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Purpose: To assess clinical outcomes in laser cataract surgery when compared to conventional phacoemulsification.

Methods: Patients electing to undergo routine cataract surgery were recruited onto this study after explanation of the study aims and signing the Ethics Committee approved informed consent form. Patients with the eye with the most visual compromise was operated on first using the laser procedure. Laser capsulotomy with or without lens fragmentation was followed by cataract surgery using the minimum phaco energy required to aspirate the lens. The fellow eye was subsequently operated on using standard phacoemulsification surgery technique as a control. Standard clinical measures were used to assess patient outcomes.

Results: 60 laser treated eyes and 45 conventionally treated fellow eyes were included in the analysis. At 3 months postoperative, the logMAR UCVA was 0.16±0.20 and 0.23±0.16 for laser and control eyes respectively. IOP was 14±9 mmHg for both groups at baseline and 13±2 mmHg at 3 months. 3 eyes in the laser group and 4 eyes in the control group had pressures over 25 mmHg at 1 day post-operatively, resolving by 1 week. 4 eyes in each group had a rise in IOP of ≥10 mmHg at day 1 resolving by 1 week. Corneal thickness was 548±39 um and 542±57 um at baseline and 531±72 um and 529±45 um at 3 months postoperative for laser and control groups respectively.

Conclusions: Early clinical data suggest that outcomes with laser cataract surgery show no significant differences in clinical outcomes. If the intended benefits of laser cataract surgery (which include more consistent capsulotomies and reduced phaco energy during lens extraction) can be realized, it appears there will be no adverse effects on clinical outcomes.

CR: K.H. Edwards; E. W. Frey; LensAR Inc; R. Naranjo Tackman; LensAR Inc, C; J. Villar Kuri; LensAR Inc; N. Quezada; LensAR Inc, E; T. Bunch, LensAR Inc, E.
Support: None CT: www.clinicaltrials.gov; NCT01011117

5395 - A411
High Intensity Focus Ultrasound (HIFU) to Breakdown Lens for Cataract Surgery
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Purpose: To study the effects of non-invasive HIFU on porcine lenses

Methods: Porcine lenses were hardened by soaking in 10% formalin (for 1.25, 5 minutes) then treated with HIFU (4 MHz transducer focused at 40±1.64 mm for 3 minutes) and compared to untreated porcine lenses (no HIFU). Temperature was kept constant at 36°C, 40°C or 46°C. The following measurements were then performed: 1) Lens hardness using the Bose System (Bose Corporation USA, MN); 2) Sound velocity through the lens (Panametrics USA, MA); 3) Phacoemulsification energy (power X time of ultrasound - Millennium, Bausch & Lomb USA, NY); 4) Transmission electron microscopy (TEM, JEOL USA, MA) to analyze HIFU-induced anatomical changes.

Results: Force (Bose measurements) was decreased in lens soaked for 2.5 minutes treated with 1.25 minutes of HIFU at 36°C, 40°C and 46°C (Table 1): Sound Velocity was significantly decreased for lenses treated with HIFU for 2 minutes and 36°C (p=0.02, p=0.03 respectively). Phaco energy was significantly reduced for lenses measured in formalin 1.25 minutes with HIFU for 2.5 minutes at 36°C. All others values were not statistically significant. Post-HIFU TEM revealed changes in the center of the lens: spreading of membrane spaces and vacuole formation. There was no damage in the lens capsule.

Conclusions: HIFU affects lens integrity primarily through mechanical shearing; temperature changes are secondary since the best results were at 36°C.

CR: L.A. Arana, None; A.G.T. Pinto, None; R. Chen, None; S. Morales, None; J. Josep, None; K. Kern, None; J.D. Barbosa, None; K. Shung, None; P. Bhadri, None; M. Humayun, None.
Support: None

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5396 - A412

Surgically-Induced Astigmatism in Phacoemulsification: 2.2mm versus Eleven Scalpel Blade Clear Corneal Incisions


Purpose: To compare clinical outcomes of coaxial small incision (2.2 mm) and coaxial standard conventional blade clear corneal cataract surgery

Methods: In a retrospective trial, 39 eyes with cataract and mild to moderate corneal astigmatism (1.35±0.75 D on IOL, master keratometry) undergoing phacoemulsification were randomized to receive coaxial surgery with 2.2 mm (group 1) or eleven scalpel blade (group 2) 2.1 to 2.3 mm corneal incisions. Main outcome measures were postoperative BCVA, corneal and refractive astigmatism, and Surgically-induced astigmatism (SIA); calculated using the Unpaired T test analysis method. Thirty seven eyes made it to the post-operative assessment at 4 and two eyes at 6 months, where they all underwent refraction, IOL master keratometry, and clinical examination.

Results: At 4 weeks follow up, the mean SIA in group 1 was -0.33±0.72 D (standard deviation 0.72) and -0.13±0.35 D (standard deviation 0.35) in group 2. Statistical analysis of our results using unpaired t-test revealed that the difference between the two groups was not significant (95% confidence Interval). The average line gain of LogMAR visual acuity was 0.41 in group 1, and 0.39 in group 2. Again, the difference in the amount of BCVA gained between the 2 groups was not statistically significant (p=0.05)

Conclusions: There is a movement underway to choose the smallest incision and type of knife in cataract surgery in order to minimize the amount of surgically induced astigmatism. However, in our study, we found the difference in the value of SIA to use 2.2 mm vs eleven scalpel blade clear corneal incisions is statistically insignificant.

Support: None

5397 - A413

Changes in Pupil Area and Dynamics Following Cataract Surgery

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Purpose: The pupillary light response is mediated by retinal rod and cone photoreceptors, as well as the recently identified melanopsin-expressing photoreceptive retinal ganglion cells (pRGCs). pRGCs are maximally sensitive to short wavelength light, the transmission of which is reduced by cataract. Here we investigate the effects of cataract surgery on the pupillary light response.

Methods: Patients undergoing cataract surgery and intraocular lens implantation with an ultraviolet-only blocking lens implant (Acrysoft, Alcon Laboratories, Inc.) had pupillometry measurements taken prior to and 2-4 weeks after surgery. The pupil of the operated eye was dilated with topical tetracaine 1%, and following dark adaptation a white light stimulus of 4 lux was provided to that eye. Measurements of the consensual response were taken with a P3000 pupillometer (Procyon). An infrared camera recorded the response at 25fps, which was then analyzed using PupilFit software (Procyon) looking at both pupil area and dynamics. Paired t-tests were carried out comparing the responses before and after surgery.

Results: In this ongoing study, 6 patients have been screened to date (4 female and 2 male), with a mean age of 77 years (range 70-86). All 6 patients showed an increased pupil constriction following surgery as measured by pupil area following 10s of light exposure (p<0.005). There was also a change in pupil dynamics with the postoperative measurements showing a more sustained constriction.

Conclusions: The change in maximal pupil constriction following cataract surgery is consistent with an increase in light transmission into the eye following removal of the cataract. The observed changes in sustained pupillary constriction may be attributed to increased pRGC stimulation due to an increase in short wavelength light transmission, as would be expected following cataract removal. This data suggest that pupillometry may provide a useful assay to study ocular function following surgical intervention.

Support: University of Oxford

5398 - A414

Expulsion Force Measurements of Ophthalmic Viscosurgical Devices (OVD)


Purpose: In cataract surgery the OVD is an important tool. The OVD maintains the ocular pressure and facilitates removal of the lens. The expulsion force was measured for several commercial OVDs. The expulsion force was the same during emptying the syringe. However, one product, a dispersive OVD, showed an early peak before going back to lower forces. Dispersive OVD 2 showed an increasing force throughout the test.

Results: The results were on average between 16 and 24 N. For most products the expulsion force was the same during emptying the syringe. However, one product, a dispersive OVD, showed an early peak before going back to lower forces. Dispersive OVD 2 showed an increasing force throughout the test.

Conclusions: There are significant differences in force required to expel OVDs from their delivery system including maximum force required and changes in force over the entire delivery.

Support: None

5399 - A415

Clinical Comparison of Ciliary Sulfus or Ciliary Body Localization in Transcleral Fixation of Posterior Chamber Intraocular Lens

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Purpose: To compare clinical outcome of ciliary sulcus or ciliary body localization in transcleral fixation of posterior chamber intraocular lens (PIOL)

Methods: Retrospective review of 65 patient’ eyes were divided into 2 groups based on the ciliary sulcus (Group 1, G1:1mm away from the limbus, N=28) or ciliary body localization (Group 2, G2: 3mm apart from the limbus, N=28) in transcleral fixation of PIOL. Visual and refractive outcomes, biometric changes, and complications including retinal detachment, dislocation or tilt of PIOL, iris capture of PIOL were compared between two groups in the mean follow-up of 29 months.

Results: Iris capture or dislocation of PIOL in G1 (14.3%, 20%) was more frequent than in G2(0%, 4.2%), which did not show a statistical significance. There is no difference of best-corrected visual acuity between two groups. Discrepancy of final refractive error from the target refractive error was -0.88 and -0.57 diopter in G1 and G2, respectively.

Conclusions: Ciliary body localization in transcleral fixation of PIOL tend to be a safe and effective procedure as an alternative option compared with ciliary sulcus localization.

Support: None
5401 - A417
Factors Determining Refractive Error and Stabilization After Cataract Surgery
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Purpose: To determine the influence of preoperative or intraoperative factors in the amount and stabilization of the refractive error after uncomplicated cataract surgery.

Methods: Random effects models were used to identify the relationship between some preoperative factors (age, lens opacity determined by LOCS grading scale, anterior chamber depth determined by Orbscan II topography, IOLMaster biometry and Otsican-1000 biometry) or intraoperative factors (surgical technique; ultrasound time, aspiration time and flow rate) and the amount of sphere refractive error after surgery. This study included 5 follow up visits: at 24 hours (V1), 1st (V2), 2nd (V3), 3rd (V4) and 4th (V5) week. The stabilization of sphere refractive error was analyzed by MANOVA.

Results: 4 eyes of 45 patients, 64.4% female, mean age 63.5±13.35 years were analyzed. Preliminary estimates were identified to significantly determine postoperative sphere refractive error: age (p<0.001), nuclear opacity (p=0.0361), anterior chamber depth (p=0.0093 with Orbscan, p=0.0023 with IOLMaster, p=0.0032 with Otsican-1000), aspiration time (p=0.0456) and flow rate (p=0.0241). We found a directly proportional relationship between final spherical refraction with all factors except with anterior chamber depth that showed an inversely proportional relationship. However, only age was related with early refractive stabilization (p=0.0330), finding a less refraction change between visits in older patients.

Conclusions: There are preoperative factors that may influence the refractive outcome and stabilization. This information could be useful to surgeon to improve the information given to patients before surgery.

CR: J.M. Herreras, None; V. de Juan, None; I. Perez, None; A. del Rio, None; A. Morejon, None; I. Fernandez, None; R. Martin, None; G. Rodriguez, None.

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5402 - A418
Changes in the Number of Cataract Surgeries in Argentinean Provinces Between 2001 and 2008
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Purpose: In developing countries aging populations are making an impact in regard to the number of cataract surgeries needed to prevent blindness or visual impairment. The purpose of this study was to compare the number of surgeries in all Argentine provinces between 2001 and 2008 to identify changes and trends with the aim of helping the authorities for better planning.

Methods: Data for cataract surgeries in the public and private sectors were supplied by one or more ophthalmologists from each province, some of whom had already participated in the 2001 study. Preliminary estimates were obtained; final estimates of the studied parameters with both devices demonstrated an overall high reproducibility. CV for AxL measurements was 0.08 (95%CI 0.11-0.04) and 0.05 (95%CI 0.06-0.04) with IOLMaster and LenStar respectively with corresponding CR of 0.2% and 1%. Agreement between average AxL measurements performed by IOLMaster and LenStar was high with a mean difference of -0.02 mm (95%CI -0.04/-0.008) and limits of agreement of -0.12 (95%CI -0.09/-0.15) and 0.07 (95%CI 0.04-0.1). Agreement between average ACD measurements was low and increased after adding CCT values to ACD measures performed by LenStar.

Conclusion: LenStar is an accurate, fast and highly reproducible instrument that could provide additional informations of interest in the clinical setting of cataract and refractive surgery.

CR: D. Schiano Lomoriello, None; F. Oddone, None; M. Parravano, None; L. Tranchina, None; P. Ducoli, None.

Support: None

5403 - A419
Effects of Intracameral epinephrine on Heart Rate and Blood Pressure During Cataract Surgery
N. Saraiya, C. Bouchard, A. Khanna. Ophthalmology, Loyola University Medical Center, Maywood, IL.

Purpose: To determine the influence of the injection of intracameral epinephrine/ lidocaine (epi-“Shugarcaine”) has any statistically significant effect on heart rate or blood pressure during cataract surgery in patients with poor pre-operative dilation or patients at risk for intraoperative floppy iris syndrome (IFIS).

Methods: This study is a prospective, observational study and includes 2 categories for intraoperative floppy iris syndrome (IFIS) interventions (sedatives, anti-hypertensives, etc.) used throughout the procedure during Cataract Surgery.

Results: Thus far with a n=13, analysis of our data using the measures analysis of variance test (with the Tukey multiple comparisons test - TexaSoft, WINKS SDA Software, Sixth Edition, Cedar Hill, Texas, 2007) demonstrates a statistically significant decrease in the systolic blood pressure between time t=1 and t=2 minutes (p=0.03). There was no statistically significant difference in patients’ heart rate and diastolic blood pressure before and after the injection of the epi-“Shugarcaine” mixture. No additional medical interventions (sedatives, anti-hypertensives, etc.) used throughout the procedure were noted.

Conclusions: Unlike previously hypothesized, intracameral epi-“Shugarcaine” used during cataract surgery in patients with poorly-dilating or poorly-stabilized pupils does not seem to adversely increase patients’ blood pressure or heart rate. It, therefore, may be safely used to stabilize and dilate the iris without concern that adverse effects on intraoperative cardiovascular parameters will ensue.

CR: N. Saraiya, None; C. Bouchard, None; A. Khanna, None.

Support: None

5400 - 5403
Reproducibility of Ocular Biometry Using Non Contact Optical Low- Coherence Reflectometer
D. Schiano Lomoriello1, F. Oddone1, M. Parravano2, L. Tranchina2, P. Ducoli2. 1Ophthalmology, Fondazione G.B.Bietti-IRCCS, Rome, Italy; 2Ophthalmology, Fondazione GB Bietti-IRCCS, Rome, Rome, Italy; 3University of Rome Tor Vergata, Rome, Italy.

Purpose: To assess the reproducibility of biometric measurements performed by LenStar and to investigate the agreement with IOLMaster in normal subjects.

Setting: Department of Ophthalmology, Fondazione G.B.Bietti-IRCCS, Rome, Italy.

Methods: Each subject underwent 3 measurement sessions either with IOLMaster and LenStar. Axial length (AxL) and anterior chamber depth (ACD) were measured by IOLMaster and AxL, ACD, central corneal thickness (CCT) and lens thickness (LT) were measured by LenStar. To assess and compare repeatability the coefficient of repeatability (CR), coefficient of variation (CV), intraclass correlation coefficients (ICC) with 95% confidence interval (CI) have been calculated. Agreement between average measurements of AxL and ACD by IOLMaster and LenStar was assessed by Bland/Altman plots.

Results: 36 consecutive healthy subjects were included in the analysis. Measures of the studied parameters with both devices demonstrated an overall high reproducibility. CV for AxL measurements was 0.08 (95%CI 0.11-0.04) and 0.05 (95%CI 0.06-0.04) with IOLMaster and LenStar respectively with corresponding CR of 0.2% and 1%. Agreement between average AxL measurements performed by IOLMaster and LenStar was high with a mean difference of -0.02 mm (95%CI -0.04/-0.008) and limits of agreement of -0.12 (95%CI -0.09/-0.15) and 0.07 (95%CI 0.04-0.1). Agreement between average ACD measurements was low and increased after adding CCT values to ACD measures performed by LenStar.

Conclusion: LenStar is an accurate, fast and highly reproducible instrument that could provide additional informations of interest in the clinical setting of cataract and refractive surgery.

CR: D. Schiano Lomoriello, None; F. Oddone, None; M. Parravano, None; L. Tranchina, None; P. Ducoli, None.

Support: None
Simultaneous Bilateral Cataract Surgery


Purpose: Evaluation of efficacy and safety of bilateral cataract surgery performed consecutively on the same day.

Patients and Methods: Retrospective study from 2005 to 2009 on patients operated of bilateral cataract surgery on the same day at the Department of Ophthalmology at the Avicenne Hospital. The surgical procedure was done as two distinct surgeries consecutively.

Results: Twenty four eyes of 12 patients have been recruited. Mean age of patients was 70 years old (from 33 to 86 years old). The main reason for operating both eyes of cataract on the same day was the difficulty of moving the patient from their place to the hospital because of a long past medical history. Twenty one eyes have been operated by phacoemulsification, 1 eye underwent extra-capsular surgery. Mean follow-up was 3 months (from 2 weeks to 7 months) and visual acuity was perfumed for all patients. Mean Visual Acuity at Baseline was at 40/200, final visual acuity was measured between 100 to 200/200 for the 21 eyes operated by phacoemulsification. One complication has been collected such as iris herno, no endophthalmy has been recorded.

Conclusion: Simultaneous bilateral cataract surgery in our study can be considered as an effective and safe procedure with limited indications.

CR: T. Grenet, None; J. Zeribh, None; A. Chassignol, None; C. Khammari Chebbi, None; S. Nghiem-Buffet, None; F. Fajnkuchen, None; C. Rohart, None; G. Chaine, None.

Support: None.

Effect of Iris Hooks on Surgically-Induced Astigmatism (SIA) After Cataract Surgery


Purpose: To select the power of an IOL it is important for the cataract surgeon to estimate the effect of the incision on SIA. If the pupil fails to dilate or the iris is floppy, the surgeon may anticipate using iris hooks to dilate and stabilize the pupil. This study was designed to estimate the effect of the incisions for iris hooks on SIA after cataract surgery with temporal 2.6 mm incision.

Methods: The IRB of Columbia University Medical Center approved this retrospective study of 194 eyes from 158 patients, with an average age of 76 years. The study included 163 eyes from 130 patients who did not need iris hooks and 31 eyes from 28 patients with iris hooks (Alcon/Grieshaber). In these eyes, the surgeon made 4 incisions in a diamond pattern in peripheral clear cornea for the insertion of iris hooks. The temporal 2.6 mm corneal incision was located directly above the temporal iris hook. At the end of the procedure, the surgeon hydrated the temporal 2.6 mm incision and each of the less than 1 mm incisions for the hooks. The surgeon measured corneal astigmatism with a manual keratometer (Bausch & Lomb) during post-operative visits at 1 day, 1 week, 1 month, 6 months, and 18 months. SIA was calculated with an online “Surgically Induced Astigmatism Calculator” (www.doctor-hill.com), at each follow-up time for both surgical groups. The two groups of data at each time interval were compared with a t-test for two independent populations. Statistical significance was defined as a p value of 0.05 or less.

Results: Mean SIA was slightly greater at all time intervals for the group with iris hooks than the group without iris hooks (Figure). However, there was no significant difference between groups at any time interval after surgery.

Conclusion: This study did not detect a statistically significant effect of the four incisions for iris hooks on SIA after cataract surgery with a temporal 2.6 mm incision.
5408 - A424
The Effect of Soft Contact Lenses on Biometry Prior to Cataract Surgery

Purpose: A case is presented of a 61 year old male who underwent cataract surgery after inadvertently undergoing biometric measurements while wearing soft contact lenses. His postoperative refraction was unacceptable and he underwent an intracocular lens exchange with good results. The current study looks at the effect of wearing soft contact lenses while undergoing measurements with the IOLMaster (Carl Zeiss Meditec, Inc., Dublin, CA). A pattern is sought that would easily alert the surgeon if this unfortunate mistake has been made.

Methods: Biometry was performed on 16 eyes from 8 healthy myopic volunteers. Each was measured while wearing their own soft contact lenses and again without the lenses. Axial length, keratometry readings, anterior chamber depths, and IOL power calculations for a plano postoperative refraction were recorded. A comparison between the two measurement groups was performed.

Results: The refractions of the volunteers ranged between -0.25 and -8.125 spherical equivalent. Axial length (AL) and anterior chamber depth were minimally changed by the use of soft contact lenses. Average keratometry (CR) readings were changed by an average of 3.6 D. The average miscalculation in IOL power was 4.22 D. The value of miscalculation was very similar to the refraction of each subject. The average AL/CR ratio for the control group was 3.18 while the average for the non-contact group was 2.92.

Conclusion: The wearing of soft contact lenses during biometric measurements will cause an error largely dependent on the change in corneal keratometry measurements. In myopic patients, it will cause the reading to be considerably flatter resulting in a miscalculation of intraocular lens power roughly equivalent to the power of the contact lenses. Outlying keratometry readings may indicate this error and deserve repeat measurement. A simple calculation of AL/CR will also help identify those that may have been measured with IOLMaster while wearing their soft contact lenses.

CR: D. Prescott, None; J.W. Cowdin, None; V. Lopez, None.

Support: None.

5409 - A425
Reliability and Reproducibility of IOLMaster Optical Biometry Measurements for Cataract Surgery Preoperative Assessment, Pre- and Post-Dilation and Examination
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Purpose: Studies have shown that dilation and examination of the eye can result in differences in biometry, leading to the belief that precise preoperative assessments should be based on a pre-examined eye. Thus, a return visit is necessary to complete the preoperative assessment. We evaluate the effects of preoperative cataract dilation, keratometry, and slit-lamp examination on IOLMaster (Zeiss) biometry, axial length, anterior chamber depth, and IOL K values, with additional evaluation of test-retest variability. The goal is to identify whether optical biometry can be performed on the initial cataract examination visit, and reduce the need for an additional visit for biometry.

Methods: 80 phakic eyes from 47 patients ages 60 and above presenting to outpatient ophthalmology clinic were recruited. All patients had IOLMaster biometry performed by the same operator before and after dilation and pre-examination analysis. Minimum slit-lamp examination was performed in all examined eyes. Pre-examined data was compared to post-examined data for each subject, using ANOVA for differences in axial length, anterior chamber depth, K1 and K2.

Results: The IOLMaster showed a high level of reproducibility between pre-examination and post-examination measurements. Mean refractive error in the examined group was .2 for the MTA80 lens compared to an unexamined group mean refractive error of .002 and control of .13. For MA30A, the mean difference in refractive error between examined and unexamined groups was .21, compared to .15 between examined and control groups. Similar results were obtained for ACI200SR. Differences in axial length, anterior chamber depth, K1 and K2 were negligible in all three groups.

Conclusions: We conclude that the difference in biometry and refractive error post-examination is minimal, and that IOLMaster measurements may be taken post-examination. This will enable clinicians and patients to minimize redundant visits by performing pre-operative biometry measurements on the initial cataract examination assessment.

CR: N.M. Shoshani, None; G.T. Liu, None; A. Shirivastava, None.

Support: None.
5412 - A428
Intraoperative Floppy Iris Syndrome and Cataract Surgery Complications Associated With the Use of Tamsulosin
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Purpose: Intra-operative floppy-iris syndrome (IFIS) is a recently described condition associated with cataract extraction in patients using different types of alpha-blockers for the treatment of benign prostatic hyperplasia (BPH). The aim of this study was to evaluate the incidence of IFIS and other intraoperative findings in patients using Tamsulosin versus controls.
Methods: A prospective case control study of 233 male patients who underwent cataract surgery. Patients were divided into 2 groups: patients on Tamsulosin (n=99) and controls (n=194). Data including age, preoperative pupil size, medical and ocular conditions, incidence of IFIS and other intraoperative complications were recorded. The surgeons used the same preoperative procedures for all patients. Preoperative pupil dilation was classified as good (≥7mm), fair (6–5mm) or poor (<5mm).
Results: Differences in the prevalence of diabetes, hypertension, glaucoma or pseudoexfoliation syndrome were not statistically different between groups. The mean age of patients was comparable for the control and Tamsulosin groups (73.4±7.6 and 75.6±6.9 years, respectively). The incidence of IFIS was higher in the Tamsulosin group (16 of 39) compared to the control group (10 of 194). These findings were statistically significant (p=0.001). In the Tamsulosin group, iris prolapse was more frequent (p=0.01) and surgeons used different pupil management techniques more often (p=0.006). However, incidence of capsular tear was very low and was not different between groups. No apparent relationship between the preoperative dilation and the occurrence of miosis during cataract surgery were noted for either group of patients.
Conclusions: Our data showed a higher incidence of IFIS and iris prolapse in patients using Tamsulosin compared to controls. Our data highlights the fact that patients using Tamsulosin require special attention during cataract surgery. The use of specific techniques is crucial to avoid intraoperative complications.
CR: S. Bakalian, None; S.K. Lindley, None; M. Elhilali, None; B.F. Fernandes, None; D. Faingold, None; M.N. Burnier, None.
Support: None.

5414 - A430
Refractive Outcome and Complications of Phacoemulsification in Eyes With Short Axial Length in Korea
Purpose: To evaluate the refractive outcome and complications of phacoemulsification in eyes with short axial length in Korea.
Methods: A retrospective analysis was performed on 57 eyes of 50 patients (axial length ≤22.0mm), 50 eyes of 41 patients (22.0mm ≤ axial length < 26.0mm ) who had undergone cataract surgery. IOL power was calculated with the SRK II, SRK/T, Binkhorst, Holladay I, Hoffer Q and Hoffer F formula. The differences between the predicted refractive error and the actual refraction were compared and analyzed. The intraoperative and postoperative complications were evaluated.
Results: The Binkhorst, Holladay I, Hoffer Q and SRK/T have similar predictive accuracy and the SRK II has lower predictive accuracy in eyes with short axial length. Hyperopic shift tends to occur with SRK II in eyes with short axial length and with Binkhorst, Holladay I and Hoffer Q in eyes with normal axial length. There were more postoperative complications such as corneal edema, postoperative anterior chamber inflammation in eyes with short axial length. In eyes with short axial length, preoperative predicted IOL power showed a tendency to be hyperopic and less accurate with SRK II and more accurate with Binkhorst, Holladay I, Hoffer Q and SRK/T.
Conclusions: In eyes with short axial length in Korea, preoperative predicted IOL power showed a tendency to be hyperopic and less accurate with SRK II and more accurate with Binkhorst, Holladay I, Hoffer Q and SRK/T. There were more postoperative complications in eyes with short axial length.
CR: R. Jun, None; Y.-E. Lee, None.
Support: None.

5413 - A429
Elevated Intraoperative Intraocular Pressure
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Purpose: To report two cases of intraoperative elevated intraocular pressure (IOP) associated with intracameral injection of either trypan blue or Shugarcane solution.
Methods: Retrospective, observational case series.
Results: Two eyes of two patients (65 yo, 5F; 70 yo, 1F) demonstrated elevated intraocular pressures during cataract surgery. Pre-operative axial length, anterior chamber depth and IOP were within normal limits for all three eyes. None of the two patients had previous surgery on the operated eye nor did they have any known ocular disease other than cataract. Both patients underwent uneventful periocular anesthesia with 0.75% bupivacaine and 2% lidocaine mixed with 1 ml dose of hyaluronidase. In both eyes, a supersharps blade was used to create a paracentesis. In both eyes, an intracameral injection of trypan blue followed by shugarcane solution in two eyes (combination of preservative free lidocaine, 1:000 epinephrine mixed with balanced salt solution) was immediately followed by a cloudy cornea and significantly elevated IOP measured by palpation and tonopen. Both eyes revealed a normal anterior chamber depth, no iris bowing, and no evidence of globe prolapse or expulsion of intraocular contents. In both eyes, the planned surgical procedure was aborted and efforts to lower the IOP was begun. In one eye, oral hypo-osmotics and topical IOP-lowering eye drops were used to sufficiently lower the IOP, and the IOP returned to normal limits and subsequent examination revealed baseline visual acuity and IOP. In the second patient, Ophthalmic cocktail and inferior cantholysis were performed, and IV Mannitol was administered in addition to topical IOP-lowering drops. An ultrasound B scan was performed and ruled out a choroidal hemorrhage. Additional paracentesis sites were performed, followed by a surgical iridectomy. Subsequent examination revealed elevated IOP and no light perception vision.
Conclusions: In our two cases, IOP became significantly elevated immediately following intracameral injection of trypan blue or Shugarcane solution. The mechanism of IOP elevation is unclear, as there were no anatomic abnormalities that could be identified. To our knowledge, this mechanism of intra-operative IOP elevation has not been reported in the ophthalmic literature and should be recognized as a sight-threatening condition.
CR: R.H. Ghafouri, None; S. Liang, None; T.K. Pira, None.
Support: None.

5415 - A431
Favourable Outcome After Cataract Surgery With IOL Implantation in Patients With Juvenile Idiopathic Arthritis Associated Uveitis
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Purpose: To investigate the outcome after minimal invasive surgery with IOL implantation and intraoperative intracameral triamcinolone injection in patients with uveitic cataract in juvenile idiopathic arthritis (JIA). The management of cataract in these patients is challenging, and IOL implantation is controversial.
Methods: Retrospective analysis after phacoemulsification with the bag IOL implantation in 16 patients (17 surgeries) with ANA-positive JIA associated chronic uveitis. We performed a 25 G capsulotomy with anterior vitrectomy and intravitreal triamcinolone (TA) injection. Mean age at uveitis onset of 5±2 years, and surgery was performed at a mean age of 11±2.2 years. Preoperatively, uveitis was inactive in all patients, and visual acuity was logMAR 0.8±0.44; additional uveitis complications were present in all patients, and were on systemic immunosuppression / biologics.
Results: After surgery (mean follow-up 15±9 months), presence of cystoid macular edema, papilledema, band keratopathy, ocular hypertension/glaucoma, and hypotony did not increase compared to baseline and to a control group without IOL implantation. By 1 year, IOL deposits were present in 4 patients, and synochiae in 9. There was no significant worsening of AC inflammation (by cell numbers and laser flare values) and no alteration of immunosuppressive therapy. The visual acuity was improved (≥ 2 lines) in all patients (mean logMAR 0.35). Retinodial membrane formation occurred in 6 patients, requiring Nd:YAG capsulotomy in 4 of them.
Conclusions: Phacoemulsification and in the bag IOL implantation may improve visual outcome in JIA associated uveitis with appropriate minimal invasive surgical technique including TA injection. A perioperative requirement is well controlled uveitis with appropriate use of topical steroids and systemic immunosuppression or biologics.
CR: R.S. Grajewski, None; B. Zurek-Imhoff, None; M. Roese1, None; C. Heinz1, None; A. Heiligenhaus2, None.
Support: None.
Changes in CDE With Laser Lens Fragmentation Compared With Standard Phacoemulsification Cataract Surgery

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Purpose: To determine if laser lens fragmentation reduces the need for phacoemulsification energy (as measured by CDE) in cataract extraction when compared to conventional phacoemulsification surgery.

Methods: Patients electing to undergo routine cataract surgery were recruited onto this study after explanation of the study aims and signing the Ethics Committee approved consent form. The eye with the most vision impairment was operated on first using the laser procedure. Laser capsulotomy and lens fragmentation was followed by cataract surgery using the minimum phaco-energy required to aspirate the lens. The fellow eye was subsequently operated on using standard phacoemulsification surgery. The amount of energy utilized in each surgery was recorded using the displayed Cumulative Dispensed Energy (CDE) in the Alcon InfiniPhac and phacoemulsification device.

Results: A total of 38 laser treated eyes and 18 conventionally treated eyes were included in the analysis. For LOC3II Grade 1 cataract, mean CDE was reduced by 26.4% while for Grade 2 the reduction was 59.1%. The biggest reduction in the maximum energy required to remove the lens was 66.4% for Grade 2 cataract (14.22 Laser vs 42.35 Phaco only) and the biggest reduction in the minimum energy required to remove the lens was 61.6% (0.04 Laser vs 2.45 phaco only) for Grade 3 cataract.

Conclusions: Early data from clinical trials suggest that laser lens fragmentation can reduce the amount of ultrasound energy required to remove the cataractous lens. A larger sample of higher grade cataracts is required to determine the optimum cutting algorithms, to refine surgical technique used in conjunction with the laser fragmentation.

CR: R. W. Frey, LensAR Inc; I: LensAR Inc; E: LensAR Inc; P. K. Edwards, LensAR Inc; E. R. Narango-Tackman, LensAR Inc; C. J. Villar Kuri, LensAR Inc; C. N. Quezada, LensAR Inc; T. Bunch, LensAR Inc; S. Boff, LensAR Inc. Support: None CT: www.clinicaltrials.gov; NCT0101117

Neural Adaptation in Patients Implanted Bilaterally With Two Different Multifocal IOLs

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Purpose: Implanting two different multifocal IOLs, one retractive and the other diffractive, in the two eyes of a patient is a widely used surgical approach. It is often referred as mixed-and-match and appears to provide patients with reasonable quality of vision for both distance and near objects. Some clinical experience suggests that visual performance in these patients tends to improve with time. The underlying causes of this improvement, with special emphasis in the possible role of neural adaptation, are evaluated.

Methods: From a population of bilaterally implanted cataract patients, we selected a group with mix-and-match (Tecnis/Rezoom, AMO) (MM_group) and another with the same type of diffractive IOL (Acrylsa, Zeiss) in each eye (DE_group). They were fully evaluated one and six months after the surgeries. Best corrected visual acuity (BCVA) at far (30 m) and at reading distance (30 cm) were measured both monocularly and binocularly. A custom method for measuring visual acuity was used with a tumbling E projected on a micro-display. The optical properties of the implanted eyes were controlled in both visits. Occular aberrations were evaluated with a Hartmann-Shack sensor and the transparency and ocular scattering were tested by using a double-pass based instrument (OQAS, Visiometrics SL) and slit-lamp imaging.

Results: In the patients finally selected (five in each group), the optical properties in the two eyes were nearly constant over the 6-months time. Aberrations and scatter were stable suggesting that the quality of the retinal image was unchanged. This assured us that there were not significant modifications in the optics over time. One month after surgery, the average values of the binocular far and near BCVA expressed in LogMAR were similar for both groups MM: 0.08 ± 0.06, DD: 0.09 ± 0.05; MM: 0.94 ± 0.06, DD: 0.08 ± 0.13. After six months, binocular distance BCVA did not change for both IOLs configurations: MM: 0.10 ± 0.08, DD: 0.07 ± 0.10. However, binocular VA for the near target improved for the mix-and-match configuration (MM: 0.17 ± 0.01) versus not for the other group with two diffractive lenses (DD: 0.10 ± 0.13).

Conclusions: Binocular near vision acuity improved after six months in patients implanted with the mix-and-match approach. In patients with two diffractive IOLs, visual acuity did not change with time. Considering that there were not optical changes and differences found between the two IOLs combinations, some particular type of binocular interaction of neural adaptation appears as the main reason for visual improvement.

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Correlation of Intraocular Staylight and Visual Acuity Before and After YAG Capsulotomy

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Purpose: Examination of visual impairment due to posterior capsular opacification, especially the correlation of intraocular straylight and vision, before and after YAG-Capsulotomy.

Methods: In a prospective binocular study (Barcelona/Spain, Salzburg/Austria), 45 eyes were examined. We tested visual acuity (Snellen) and intraocular straylight (C-Quantray, straylight parameter [log(s)]) before and two weeks after YAG-Capsulotomy under photopic conditions. In each case the straylight testing was performed in the morning and with dilated pupils.

Results: Patients with posterior capsular opacification had a lower vision (0.52 log vs 0.2) and higher straylight values (1.52 log(s) ± 0.49). Comparing the straylight values with dilated pupils (1.69 log(s) ± 0.53) to the values in myosis, there was an even higher significant increase of straylight. After performing a YAG-Capsulotomy there was a significant increase of visual acuity (0.86Sn ± 0.17), asswell a significant advancement of the straylight values in myosis (1.51 log(s) ± 0.21). Unexpectedly there was no post OP significant advancement of the straylight value in dilated pupils (1.56 log(s) ± 0.22).

Conclusions: Performing a YAG-Capsulotomy leads to a significant advancement of visual acuity and therefore to more patient satisfaction. It leads aswell to a significant decrease of intraocular straylight and so to less glare. Both, pre- and postoperatively, the straylight values show a significant increase, when the pupil is dilated. This may happen due to occurrence of spherical and chromatic aberrations.

CR: P. P. Marvan, None; M. Rasp, None; T. Rückl, None; R. Michael, None; A. Dexl, None. Support: None
Prolonged Duration of Visual Recovery With Increased Rate of Poor Visual Acuity in the Diabetic Population Compared With Non-Diabetics After Cataract Surgery

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**Purpose:** To determine if there is an increased length of recovery and increased incidence of poor visual outcome in diabetic patients despite having uncomplicated cataract surgery and uneventful post-operative course compared to non-diabetic controls.

**Method:** Retrospective chart review of 577 consecutive cataract surgeries performed on 674 patients at Cooper University Hospital between January 3, 2003 and December 22, 2006. All cases were performed by resident surgeons supervised by one attending physician. Patients were divided into two groups, diabetic or non-diabetic. Final visual outcome was determined as best corrected vision of at least 20/40 vision.

**Results:** A total of 71 cases with intraoperative complications were reported and were thus not included as part of this study. In addition, development of post-operative CME was excluded. As a result, there were 165 uncomplicated cases involving diabetic patients and 238 cases in non-diabetic patients. Of the diabetic patients that achieved a best corrected visual acuity of at least 20/40 did so on average in 18.5 days with a median of 7 days. The non-diabetic group reached 20/40 on average of 16.7 days with a median of 7 days. 19 of 165 or 11% of diabetic patients never achieved a visual acuity of 20/40 or better, while only 7 of 238 or 3% of non-diabetics did not meet this goal.

**Conclusions:** By virtue of being diabetic, there is a small but significant increased in recovery time to reach an acceptable final visual acuity set at 20/40 best corrected vision. This is despite not developing CME during the post-operative period. Preexisting diabetic retinopathy may have played a role in final visual outcome. Counseling of diabetic patients on recovery time and realistic visual goals should be discussed in advance of cataract surgery.

**CR:** A.G. Chun. None.

**Support:** None.

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The Effects of Topical Coenzyme Q After Cataract Surgery: A Clinical and Confocal Study


**Purpose:** To evaluate the postoperative effects of topical coenzyme Q in patients who underwent cataract surgery.

**Methods:** 30 consecutive patients who underwent uneventful cataract surgery (3.2-3.3 mm temporal incision, phacoemulsification + IOL in the bag) were treated with topical antibiotics and corticosteroids for 2 weeks after surgery and, thereafter, with topical coenzyme Q or saline solution twice daily for 3 months. Patients received full eye examination and the following examinations before surgery and at days 5, 14, 30: Schirmer test (ST), Break-up time (BUT), aestesiometry (AE), in vivo confocal microscopy of the cornea. The density of the sub-basal nerves was calculated in the central (CND) and temporal (TND) cornea.

**Results:** Before surgery ST was 12±3 mm, BUT 1±2 sec, AE 58±2 mm, CND 7.1±2.6, TND 6.0±2.4. At day 14, a small decrease of ST, BUT, AE was found (12±1 mm, 1±1 sec and 8.3±3 mm respectively; P>0.05 compared with baseline), whereas a significant reduction in CND (2.3±2.1, P<0.003), and TND (2.4±3.1, P<0.001) was shown. At day 90, ST, BUT, AE were similar compared with baseline (P>0.05), whereas there were higher CND (6.6±3.1) and TND (5.0±3.1) compared with day 14 (P<0.01 and 0.05 respectively).

**Conclusions:** Our results confirm that even uneventful cataract surgery leads to relevant changes of the corneal nerves which may influence corneal surface function. From our short-term data, a 3-month treatment with topical coenzyme Q had a positive effect in restoring the normal anatomy of the sub-basal nerve plexus. Longer follow-up and comparison with controls are needed to provide confirmation of these findings.

**CR:** P. Fogagnolo, None; G. Ceresara, None; R. Paderni, None; P. Lapadula, None; L. Rossetti, None; N. Orzalesi, None.

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Influence of Corneal Spherical Aberration and Intracorneal Lens Asphericity on Optical Quality After Cataract Removal

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**Purpose:** To investigate the influence of corneal spherical aberration and intraocular lens (IOL) asphericity on optical quality after cataract removal.

**Methods:** We included 119 eyes of 72 cataract patients who were operated on with different spherical and aspheric IOL. 26 eyes received AcrySof SN60AT (Alcon, spherical aberration [SA] = 0.2μm), 21 eyes Sensar AR40e (AMO, SA = 0.1μm), 25 eyes Akreos TL M60 (Bausch & Lomb, SA = 0μm), 26 eyes AcrySof IQ SN60WF (Alcon, SA = 0.2μm), and 21 eyes Tecnis Z9000 (AMO, SA = -0.27μm), all IOL-SA according to manufacturer’s information. Absolute values of corneal and ocular higher order aberrations were measured, and best-corrected visual Strehl ratio based on the optical transfer function (BCVSOTF) values were computed. All trial-specific measurements (corneal topography, wavefront sensing) were performed 3-6 months after surgery. Linear regression, ANOVA and Tukey HSD test were used to evaluate intergroup differences.

**Results:** Incision size (IS) ≥ 3mm caused a significantly higher change of corneal SA (p<0.001) and showed high variance of ocular SA of 0.64±0.23μm (AR40e) and 0.32±0.34μm (SN60WF), whereas IS of 1.7 mm and 2.5 mm lead to minimally variant ocular SA of 0.24±0.07μm (MI60), 0.63±0.07μm (SN60WF) and 0.53±0.07μm (SN60AT). A significant relationship between ocular SA and BCVSOTF was shown (r=0.244, p<0.01). Smaller values for ocular SA were associated with higher values for BCVSOTF. BCVSOTF was of interindividual variance in all groups ±1e-04.28 (IOL-SA = 0.2μm), -1.04±0.16 (IOL-SA = 0.1μm), -1.02±0.17 (IOL-SA = 0μm), -0.73±0.21 (IOL-SA = -0.2μm), -1.17±0.28 (IOL-SA = -0.27μm) with significantly better values for BCVSOTF compared to all other groups (p<0.01) for IOL-SA of -0.2μm.

**Conclusions:** Because of variance of surgery induced corneal SA, predictability of resulting ocular SA is limited depending on IS. Up to minimum ocular SA of about 0.0μm, lower residual ocular SA causes better performance in terms of BCVSOTF. A distinct customization based on individual pre-surgical corneal SA is not to be postulated, but an IOL-SA of -0.2μm in addition to an incision size limited to 2.5mm shows best performance.

**CR:** T. Kohnen, None; C. Hofmann. None.

**Support:** None CT: www.clinicaltrials.gov
Use of Control Charts to Monitor Post-Cataract Surgery Acute Endophthalmitis in Spain

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Purpose: To employ statistical process control methods to determine if the rate of post-operative endophthalmitis following cataract surgery at a single institution is in statistical control.

Methods: Data on all patients who underwent cataract surgery between January 2004 and December 2008 at the Hospital de León was prospectively collected. Utilizing statistical process control charts, we analyzed the rate of endophthalmitis in these patients in order to detect trends in variation of the rate of endophthalmitis over time. The software was used to construct: (1) g-charts of the days between endophthalmitis cases in the hospital; (2) run charts of the rate of cases of endophthalmitis over time.

Results: A total of 5013 cataract surgeries were performed. The run chart illustrated a mean annual incidence of endophthalmitis of 0.18%, although in 2005 a markedly higher incidence of 0.51% was documented. Use of the g-chart revealed that over this 5-year time period, the mean number of days between endophthalmitis cases was 141 ± 2 (P<0.05), high IOP did not preclude the possibility of wound leakage. Wound leak cases did not differ significantly in terms of visual acuity, anterior chamber grading of inflammation, or presence of corneal edema. One case of endophthalmitis was identified at POD 4, which did not have a wound leak at POD 1, although the clear corneal incision was enlarged from 2.75mm to 4.1mm intraoperatively.

Conclusion: Seidel testing for wound instability has a limited role in prognostication of post-operative complications, namely, endophthalmitis. Additional assessment with blinking and globe compression did not yield any additional helpful information. A new modality of post-operative testing is needed to assess wound stability to better stratify risk of post-operative complications for improved preventative treatment.

Support: None

Intraocular straylight is a useful tool in the assessment of PCO. It measured intraocular straylight and visual acuity.

To study the correlation of posterior capsule opacification (PCO) with a wide range of clinically relevant flow and vacuum settings.

Methods: Viscoadaptive Healon5 and non-adaptive (Viscoat and Vitrax II brand) OVDs were stained with fluorescein, 6 µg/ml, for detection purposes. Each step in the surgeries was monitored by Scheimpflug photographs using the Pentacam HR (Oculus). ACD was measured before surgery, after injection of OVD, and after phacoemulsification with fluorescein, 6 µg/ml, for detection purposes. Each step in the surgeries was monitored by Scheimpflug photographs using the Pentacam HR (Oculus). ACD was measured before surgery, after injection of OVD, and after phacoemulsification (Sovereign Compact system, AMO) and presented as relative ACD of each eye compared to ACD before surgery. OVD retention is presented as OVD layer thickness after phacoemulsification relative to the ACD of each eye. Three different phacoemulsification flow and vacuum settings were used (low, medium, and high). Results were grouped according to product used at each step of the surgical procedure. The non-parametric Kruskal-Wallis test was used to evaluate differences between the medians of relative ACDs and retention of the products at each step in the surgeries.

Results: The viscoadaptive Healon5 OVD is retained in the anterior chamber during phacoemulsification the Pentacam Scheimpflug photographs allow us to objectively demonstrate that the viscoadaptive Healon5 OVD is retained in the anterior chamber during phacoemulsification at all settings tested. Healon5 OVD is retained to an equal or greater extent than the dispersive Viscotat and Vitras II OVDs.

Conclusions: The viscoadaptive Healon5 OVD has the same retention capacity in the anterior chamber during phacoemulsification as the dispersive OVDs tested over a wide range of clinically relevant flow and vacuum settings.

Support: None
Posterior Capsule Staining With Tryptan Blue During Cataract Surgery in Patients With A Peripheral Iridotomy

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Purpose: To compare the effectiveness, cellular toxicity, and cost implications of disposable sterile surgical marker pen ink (Tetran violet) and commercial Trypan blue in staining of anterior lens capsule in cataract surgery.

Methods: The ink cartridge of Aspen Sterile Surgical Marking Pen was first removed and the ink was extracted by irrigating balanced salt solution (BSS) through the cartridge. The resulting ink solution was then used to compare with commercially available Trypan blue capsular staining solution and BSS was used as a control. Cultured corneal epithelial cells were used to evaluate toxicity of the marker ink while commercial available trypan blue was used as control. Human lens capsules (obtained from eye bank eyes) were used to evaluate the effectiveness of capsular staining. Cultured corneal epithelial cells were incubated with pH adjusted marking pen ink, Trypan blue and BSS at fixed durations and toxicity were measured with Phase contrast microscope (Nikon TE200 Inverted Microscope, Nikon USA, Melville, NY). Furthermore, the cost of ink pen and filter straw with commercially available Trypan blue staining solution were compared.

Results: pH adjusted disposable marker ink solution showed similar cytotoxicity profile in comparison to Trypan Blue. The effectiveness of capsular staining of the marker ink is also similar to that to Trypan blue. The cost of the marking pen ink is only a fraction of the commercially available Trypan blue preparation.

Conclusions: Surgical marking pen ink can be easily prepared in the OR and may be used as a safe and inexpensive alternative to Trypan Blue in visualization of anterior lens capsule during cataract extraction. This approach may be particularly useful in developing countries.

CR: L. Yang, None; S. Kanayama, None; S. Garty, None; T. Shen, None.
Support: None
Acylation Reactions Soften and Liquify Cataracts: A Novel Method to Facilitate Cataract Surgery

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Purpose: To demonstrate the ability of acylation reactions to sever the abnormal crosslinks leading to cataract formation. These reactions solubilize lens proteins so the cataract can be dispersed, liquified and removed by aspiration. This is accomplished by replacing the positive (NH₃) charge with a chemical moiety that exhibits a negative or neutral charge on deprotonated free amines and reduces the pKa to a more acidic level thereby rendering the proteins more soluble at a neutral pH.

Methods: Explanted rabbit lenses were exposed to UV light to induce cataract formation. A needle delivery system (which adds the proper amount of treatment solution) was developed to inject the reagents directly into the lens nucleus. This was also performed on explanted human cataractous lenses ranging from grade 3 to grade 4. Injection of specific acylation agents followed pre-treatment injections with a slightly alkaline buffer solution to first deprotonate free amines.

Results: Injections of acylation compounds into the lens nucleus resulted in complete dispersion and partial liquification of the UV induced rabbit cataracts and grade 3 human cataracts. Grade 4 human cataracts were partially dispersed after acylation treatment.

Conclusions: Injection directly into the lens nucleus during cataract surgery can soften and liquify cataracts and may permit removal by simple aspiration. Since cataract is the leading cause of world blindness, this compound may be of tremendous importance in simplifying cataract surgery.

CR: R.A. Eiferman, Patent filed, P; D.P. DeVore, patent filed, P.
Support: KY Enterprise Fund
5433 - A449
Complication Rate and Risk Factors for Intraoperative Complications in Resident-Performed Phacoemulsification Surgery

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Purpose: To analyze the outcomes of the first 100 phacoemulsification surgeries performed by residents and to identify risk factors for intraoperative complications.

Methods: Retrospective chart review of the first 100 performed phacoemulsification cases of four consecutive residents at the Department of Ophthalmology, Regensburg University Medical Center, Regensburg, Germany.

Results: A total of 400 cases was analyzed. Posterior capsule rupture with vitreous loss was observed in 18 cases (4.5%). In 18/18 cases with vitreous loss a dropped nucleus had to be removed by pars plana vitrectomy. Risk factors for posterior capsule rupture were mature + nuclear sclerotic cataracts (6/18), pseudoxfoliation syndrome (2/18), shallow anterior chamber in hypermetropia (3/18) posterior synchia (1/18), and zonular pathology (3/18). In 12/25% posterior capsule rupture with vitreous loss occurred in eyes without risk factors. An intracocular lens was implanted in all 400 cases during surgery. In 9.25% the intracocular lens was implanted in the sulcus.

Conclusions: Residents performing phacoemulsification surgery achieved a low overall rate of major complications in eyes without risk factors. However, phacoemulsifications in cataracts with specific features, such as mature nuclei and zonular pathology should be performed by more experienced surgeons.

CR: W. Herrmann, None; A. Briszi, None; H. Helbig, None.
Support: None.

5434 - A450
Effect of Sleep Deprivation on the Performance of Simulated Anterior Segment Surgical Skill


Purpose: To measure the effect of acute sleep deprivation on anterior segment surgery skills by using a surgical simulator.

Methods: Nine ophthalmology residents (3 per training year) were prospectively evaluated by using the EYES surgical simulator on 3 occasions: pre-call (6.75 hours sleep in previous 24 hours), post-work (8 hour work day; 27 hours sleep in previous 24 hours), and post-call (<3 hours sleep in previous 24 hours). Participants kept logs to record sleep hours. The degree of sleepiness was assessed by using the Epworth Sleepiness Scale (ESS). Caffeine consumption was restricted 12 hours prior to testing. Technical skill was assessed by using the EYES anti-tremor and forces training modules and recorded on a 100-point performance scale. Performance data was compared by using a 3-factor repeated measures analysis of variance.

Results: Mean hours of sleep was less in post-call residents (1.9 ± 1.2 hours) when compared to pre-call (7.6 ± 0.6 hours, P<0.001) and post work (7.0 ± 0.4 hours, P<0.001).

Similarly, degree of sleepiness (mean ESS) was increased in post-call residents (14.7 ± 4.0) when compared to pre-call (2.7 ± 1.2, P<0.001) and post work (4.9 ± 3.2, P<0.001).

No difference in technical performance was detected by using the anti-tremor module in pre-call (85 ± 20 points), post-work (80 ± 24 points), and post-call (81 ± 26 points; P=0.51) residents or by using the forces module in pre-call (99 ± 1 points), post-work (98 ± 4 points), and post-call (98 ± 5 points; P=0.11) residents.

Conclusions: Call-associated sleep deprivation was not associated with increased technical errors in the performance of simulated anterior segment surgical skills.

CR: J.C. Eric, None; E.A. Eric, None; M.A. Mahr, None.
Support: Research to Prevent Blindness, New York, NY; Robert W. Raller Career Development Award, Mayo Foundation, Rochester, MN.

5435 - A451
Computer-Based Simulation of Cataract Surgery: Toward a New Teaching Paradigm


Purpose: cataract surgery is the most frequently performed procedure in ophthalmology and presents a significant vector of progress in the near future. Current training methods are based on an apprenticeship model which has two principal limitations: training periods depend on the availability of the instructor, and higher risk of complications due to the inexperience of the operator. Yet, recent reports have demonstrated the added value of computer-based training systems. In the context of cataract surgery, we believe a computer-based simulator could enable new teaching approaches and help assess the level of proficiency of medical residents.

Methods: This work is the result of a collaboration with researchers in the field of computer science. Through this collaboration, we have developed computer models of the main anatomical structures of the eye as well as biomechanical models of the structures manipulated during the simulated procedure. Real time computation of the behavior of the virtual eye has been made possible by developing specific algorithms and computational models. The complete simulation system consists of a computer, for computing and rendering the virtual eye, as well as devices allowing interactions with the virtual environment. Among the available interfaces is a tracking system that transmits the position of mock surgical instruments and stereoscopic glasses that provide a visual feedback similar to what can be seen through a microscope. The different equipments are integrated in a whole body mannequin and a refurbished microscope to provide added realism and a higher level of immersion of the simulation.

Results: We have simulated the main stages of cataract surgery, namely capsulorhexis, phacofragmentation and aspiration of the fragments, injection of the IOL and its deployment in the capsule.

Conclusions: With respect to the state of the art, our simulation offers similar or better realism of the capsulorhexis and phacoemulsification stages than prior work. In addition, it includes a real-time modeling of IOL injection and deployment. This step has not, to our knowledge, been addressed previously. The use of a mock up surgical unit, combined with tracking interfaces for the instruments, helps provide realistic training conditions. This allows medical resident to learn the key steps of cataract surgery, using an operating microscope while familiarizing themselves with hand-foot coordination.

CR: N. Bouchichi, None; S. Cotin, None; O. Comas, None; J. Roy, None; M. Sanz, None; C. Duriez, None; J. Dequidt, None; J. Allard, None; J. Rouland, None.
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5436 - A452
Senior Resident Phacoemulsification Learning Curve in Brazil

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Purpose: To analyse the outcomes of surgeries performed by senior residents in the learning curve related to intraoperative complications and staff interventions.

Methods: A prospective study of the phacoemulsification surgeries performed by senior residents (3rd year) in the first three months of experience with this technique at the HC-FMUSP. The intraoperative complications and the necessity of staff interventions were measured.

Results: It was included 261 surgeries. There were 30 cases of intraoperative complications, a rate of 11.54%. Major complications, that could affect final results of the surgery, markedly posterior capsule rupture and vitreous loss, had a incidence rate of 8.05% and 6.13%, respectively. The surgery was converted to extracapsular extraction of the cataract in 3 cases and in 2 cases it was needed pars plana posterior vitrectomy. There was necessary staff doctor’s intervention in 11 cases (4.22%), most of them in the first 40 surgeries.

Conclusions: With proper training and supervision, senior residents can achieve an acceptable rate of complication. Adequate supervision is essential to guarantee the surgeries results, specially in the first 40 cases, that shows the greater complications rates.

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Support: None.
5437 - A453
Accuracy of Manual Keratometry, IOL Master, and Immersion A-Scan by Inexperienced Users
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Purpose: To compare the accuracy of manual keratometry (K) versus IOL Master K for first time users. To compare the accuracy of immersion A-scan measurements with IOL Master axial length measurements for first time users. To compare the IOL choice accuracy when manual K and immersion A-scan are used versus IOL Master for first time users.

Methods: A 30 minute tutorial was given to five first year ophthalmology residents on the IOL. Master, manual K, and immersion A-scan. No further instruction was provided unless necessary to complete the task; exemptions were recorded. Residents performed IOL Master, manual K, and immersion A-scan measurements on both eyes of their partner or a volunteer. The head technician took measurements on the same eyes. An identical workshop was held with the following year’s first year residents. This time, residents measured one of their partner’s eyes. In total, measurements were taken by 10 beginning residents on 15 eyes. Results by residents were compared to those of the lead technician (standard error of measurement ± 0.18 D). Results for first year residents were compared to fifth year residents (p=0.007). For each method, timing (average and standard deviation) was recorded and defined as the time from initial instrument-to-eye contact to obtaining the ax length scan that provided a standard deviation of 0.05 or less.

Results: There was no statistically significant difference between the axial lengths measured amongst SonoMed contact manual and any Eye Cubed automated measurement (contact p=0.25, immersion p=0.29). For each method, timing (average and standard deviation) was the following: SonoMed contact manual (97.2 ± 11 sec), Eye Cubed contact manual (178 ± 11.5 sec), Eye Cubed contact automatic (156.6 ± 90.8 sec), Eye Cubed immersion manual (7.5 ± 4.6 sec), and Eye Cubed immersion automatic (47.2 ± 61.5 sec). Compared to SonoMed’s contact manual mode, the Eye Cubed provided a statistically significant improvement in measurement timing using the manual modes of contact (p=0.0004) and immersion (p=0.0002). There was no statistically significant improvement in timing using the automated measurement modes with either contact (p=0.11) or immersion (p=0.10).

Conclusions: Our study suggests that the Eye Cubed axial lengths are comparable to those obtained by SonoMed. Moreover, Eye Cubed’s most reliable setting, immersion manual, provides accurate axial lengths in one tenth of the time compared to the SonoMed and can significantly improve the efficiency of a resident clinic.

CR: M. Liu, None; R.U. Desai, None; B. Enriquez, None; E. Smith, None.
Support: None

5438 - A454
A Comparison of the Accuracy and Efficiency of an Innovative Ultrasound Device With Routine A Scan Biometry in a Resident Clinic Setting

Purpose: To evaluate the accuracy and efficiency of axial length measurements by the Eye Cubed A-scan in contact manual, contact automatic, immersion manual, and immersion automatic modes in comparison to the Sonomed 5500 A-scan in contact manual mode.

Methods: The axial length of 30 eyes from 15 patients with cataracts was measured using 5 different techniques by a single operator: Eye Cubed A-scan in contact manual, contact automatic, immersion manual, and immersion automatic modes and Sonomed A-scan in contact manual mode. The time required to complete each technique was recorded and defined as the time from initial instrument-to-eye contact to obtaining of the 5th axial length scan that provided a standard deviation of 0.05 or less.

Results: There was no statistically significant difference between the axial lengths measured amongst SonoMed contact manual and any Eye Cubed automated measurement (contact p=0.25, immersion p=0.29). For each method, timing (average and standard deviation) was the following: SonoMed contact manual (97.2 ± 11 sec), Eye Cubed contact manual (178 ± 11.5 sec), Eye Cubed contact automatic (156.6 ± 90.8 sec), Eye Cubed immersion manual (7.5 ± 4.6 sec), and Eye Cubed immersion automatic (47.2 ± 61.5 sec). Compared to SonoMed’s contact manual mode, the Eye Cubed provided a statistically significant improvement in measurement timing using the manual modes of contact (p=0.0004) and immersion (p=0.0002). There was no statistically significant improvement in timing using the automated measurement modes with either contact (p=0.11) or immersion (p=0.10).

Conclusions: Our study suggests that the Eye Cubed axial lengths are comparable to those obtained by SonoMed. Moreover, Eye Cubed’s most reliable setting, immersion manual, provides accurate axial lengths in one tenth of the time compared to the SonoMed and can significantly improve the efficiency of a resident clinic.

CR: M. Liu, None; R.U. Desai, None; B. Enriquez, None; E. Smith, None.
Support: None
Assessing the Validity of the 2010 PQRI Post Cataract Visual Acuity Measures: A Comparison of Post Operative Acuity Between Experienced and Less Experienced Resident Cataract Surgeon Cohorts


Purpose: The 2006 Tax Relief and Health Care Act established the Physician Quality Reporting Initiative (PQRI), whose premise is that higher quality care should be reimbursed more. A new 2010 PQRI outcome measure asks surgeons to report if “20/40 or better visual acuity within 90 days following (cataract) surgery” is achieved. The purpose of this study is to assess the efficacy and validity of this measure as a proxy for quality delivery of cataract surgery.

Methods: A retrospective review was conducted analyzing the post-operative outcomes of cases performed by two cohorts of resident cataract surgeons with disparate operative experience. The first cohort consisted of four third year residents (16 cases) and the second cohort included seven cases where co-morbid conditions may have affected post-operative outcomes (i.e. diabetic macular disease, other macular pathology). 11 cases were from the first cohort and 7 from the second cohort. Both groups were included in the final analysis. The cases were evaluated utilizing the 2010 PQRI measure of “20/40 or better visual acuity within 90 days.”

Results: Of the 18 included cases performed by the first cohort of May-June resident surgeons, 48 (100%) of the cases achieved 20/40 visual acuity within 90 days post-op, while 31 (91.2%) of the 34 cases performed by the July-August resident surgeons achieved this acuity. A Fisher’s test comparing the two cohorts gave a p-value of .0675, favoring to show statistical significance.

Conclusions: The “20/40 or 90 days” acuity measure was unable to show a statistically significant difference between the post-operative outcomes of July-August resident surgeons and May-June resident surgeons. Additionally, the fact that 100% of the May-June resident cases evaluated achieved this PQRI outcome measure raises the question of whether this measure will have sufficient sensitivity to differentiate cataract outcomes with the increasing complexity of the cases. Future research should study this finding as future analysis will be needed before this measure can be utilized as an accurate gauge of quality healthcare delivery.

Support: None

Evaluation of 5 Intraocular Lens Power Prediction Formulas Early in Resident Surgical Training

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Purpose: To evaluate the accuracy of five intraocular lens (IOL) power prediction formulas in early resident surgical cases.

Methods: Retrospective review of cataract surgeries performed between 12/12/2007 and 12/31/2009 by resident surgeons at a teaching hospital. Inclusion criteria were used: IOL placed in the capsular bag, post op acuity better than 20/100, available preoperative biometry, and those with at least 3 months follow up after surgery. Using preoperative keratometry (Ks) axial length (AxL) and anterior chamber depth (ACD) data, predicted postoperative refractive error (PPOR) was calculated with the Haigis (H), SRK II (S2), SRK/T (ST), Holladay I (H1), and Hoffer Q (HQ) formulas. Actual postoperative refraction (POR) was compared to PPOR. Descriptive statistics were assessed. Paired t-test and multiple regression analysis were performed.

Results: Thirty-six residents were categorized early in their learning curve. Calculations were performed by 8 third year residents. AxL ranged between 21.1 and 28.2 mm with 86% of cases between 22 and 24.99 mm. Mean difference (POR-PPOR) was -0.77±1.26 (P<0.001); it was 16.3 with PCIOL implantation (95% CI 4.7-57.7; p<0.001); it was 97.7 with AIOL implantation (95% CI 15.7-677; p<0.001); and it was 39.1 when using H1. The odds ratio of having a BCVA < 20/40 in a complicated phaco surgery was 41.5 when SIOL implantation was performed (95% CI 15.4-112.1; P<0.001) and 16.3 with PCIOL implantation (95% CI 4.7-57.7; p<0.001). It was 97.7 with AIOL implantation (95% CI 15.7-677; p<0.001); and it was 39.1 when using H1.

Conclusions: In resident-performed phaco surgeries complicated by posterior capsule rupture, good outcomes are achievable, but there is a significantly higher rate of final visual acuity being <20/40.

Support: None

Evaluation of Resident Performed Cataract Surgery Learning Curve by Complication Rates

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Purpose: Cataract is one of the most commonly performed surgeries in the world, with over 1 million procedures performed per year in the United States alone. In 2002, a resident survey revealed that the number of phacoemulsification procedures performed by residents had increased from 50 to 100; the following year, it was reported that a minimum of 80 procedures was required to achieve statistical significance in complication rates. The aim of this study was to identify a case number for which a statistically significant decline in complication rates occurs.

Methods: Retrospective chart review of resident performed phacoemulsification procedures during a 6 year period at The VA Hospital, Tennessee Valley Healthcare System in Nashville, TN. The surgeries were categorized based on the number of resident procedures performed per surgeon. The cases were divided into groups spanning every 20 procedures. A Statistical analysis using chi square was performed to determine if a statistically significant decrease in intraoperative complications in resident performed phacoemulsification cataract surgery occurred. This study suggests improvement in complication rates of resident performed phacoemulsification cataract surgery between 41-80 procedures, specifically posterior capsular rupture.

Results: 1442 cases were performed during the time period by 19 different resident surgeons. For the purpose of complication rate analysis, the cases were divided into groups spanning every 20 procedures. A Statistical analysis using chi square was performed to determine if a statistically significant decrease in intraoperative complications in resident performed phacoemulsification cataract surgery occurred. This study suggests improvement in complication rates of resident performed phacoemulsification cataract surgery between 41-80 procedures, specifically posterior capsular rupture.

Support: None

Visual Outcomes in Eyes Undergoing Resident Performed Phacoemulsification Cataract Surgery Complicated by Posterior Capsule Rupture


Purpose: Although the complication rate for resident-performed cataract surgeries range from 2.0-14.7%, it is thought that good visual outcomes can still be achieved in these eyes. The purpose of this study is to determine which intraoperative factors may have an effect on visual outcomes in resident-performed phacoemulsification cataract (phaco) surgeries complicated by posterior capsule rupture.

Methods: This is a retrospective cohort study of all of the phaco surgeries performed by residents at the San Francisco General Hospital between July 1, 2007 and September 30, 2009. Cases were identified by reviewing operative notes and clinic notes of patients that developed posterior capsular rupture during phaco surgery. Eyes were excluded if they had less than one month of post-operative follow-up. The intraoperative techniques used, as well as final best corrected visual acuity (BCVA) were recorded. BCVA was also compared to historical visual outcomes from our institution of resident performed phaco surgeries where 5% (13/267) of uncomplicated resident performed cataract surgeries had a BCVA < 20/40.

Results: During the study period, 326 resident phaco surgeries were performed, with 47 cases (8.9%) complicated by posterior capsule rupture. Forty-five of the 47 eyes had less than one month of post-operative follow-up. Twenty-five of these 45 eyes (55.6%) had sulcus intraocular lens (SIOL) implantation. Of these 25 eyes, 17 (68%) had a BCVA < 20/40. Eleven out of the 45 eyes (24.4%) had posterior chamber intraocular lens (PCIOl) implantation in the bag with 5 of the 11 (45.5%) having a BCVA < 20/40. Six of the 45 eyes (13.3%) had anterior chamber intraocular lens (ACIOL) implantation, with 5 of these six eyes (83.3%) having a visual acuity < 20/40. The remaining 3 out of 45 eyes (6.7%) were left aphakic, with 2 of the 3 eyes (66.7%) having a BCVA < 20/40. The odds ratio of having a BCVA < 20/40 in a complicated phaco surgery was 41.5 when SIOL implantation was performed (95% CI 15.4-112.1; P<0.001) and 16.3 with PCIOL implantation (95% CI 4.7-57.7; p<0.001); it was 97.7 with AIOL implantation (95% CI 15.7-677; p<0.001); and it was 39.1 when using H1.

Conclusions: In resident-performed phaco surgeries complicated by posterior capsular rupture, good outcomes are achievable, but there is a significantly higher rate of final visual acuity being <20/40.

Support: None
5446 - A462
IOL Power Calculations Using Aphakic Spherical Equivalent

Purpose: To determine if accurate IOL power calculations can be done utilizing the intraoperative aphakic spherical equivalent (IASE) data obtained with the WaveTec Vision ORange system.

Method: IOL power calculations require the keratomical power (K) of the cornea, the axial length (AL) of the eye, the expected lens position (ELP) of the IOL once implanted into the eye and the desired post operative refraction. Traditional IOL power calculation methods utilize K-values, AL determined by either ultrasound or partial optical coherence tomography and one of several IOL power calculation formulas which modify the manufacturers lens constant to an ELP for the particular IOL that was implanted. This value was compared to the 30 day manifest refraction spherical equivalent to determine the ORange method prediction error. The absolute value prediction error for the 125 eyes was 0.49 D +/- 0.35 D. We further selected a subset of 18 eyes implanted with the same IOL (Alcon SN60WF) and placebo treated eyes and used this data in a regression formula that included not only the aphakic refraction spherical equivalent but also the white-to-white (corneal diameter) measurement. For this subset, the ORange absolute value prediction error was 0.18 D +/- 0.16 D. Conclusion: Utilization of IASE measured during cataract surgery provides an effective basis for calculating IOL power.

Support: None

5447 - A463
Negative Dysphotopsia Occurrence With Toric IOLs and Its Management
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Purpose: Toric Intraocular lenses (IOLs) have become a popular form of correcting residual corneal astigmatism at the lenticular plane. In general these lenses are very well tolerated and have been well received by patients. In our review we attempt to describe a specific visual complaint of patients with Alcon Toric IOLs and how it was successfully relieved through surgical management.

Methods: Charts of patients with Alcon Toric IOLs were reviewed for complaints of temporal shadows associated with shaped lens. These patients then had their postoperative data analyzed up to the point of symptom resolution.

Results: 2 patients previously implanted with Alcon Toric IOLs (SN6AT4 and SN60T5) were noted to have negative dysphotopsias (temporal crescents with associated shimmering lights). The treated eye was immobilized without anesthesia. No retinal damage was found in Dutch Belted rabbits at 8 times the retinal exposure used for clinical settings.

Conclusions: OCT-guided femtosecond laser cataract surgery greatly improves precision and reproducibility. The laser produces sharp-edged continuous capsular cuts while lens treatment simplifies phacoemulsification, especially with dense cataracts. The treated eye is immobilized without amurosis. No retinal damage was noted to have negative dysphotopsias (temporal crescents with associated shimmering lights) or negative dysphotopsias (temporal crescents with associated shimmering lights). The treated eye was immobilized without anesthesia. No retinal damage was found in Dutch Belted rabbits at 8 times the retinal exposure used for clinical settings. However, in our review, we attempt to describe a specific visual complaint of patients with Alcon Toric IOLs and how it was successfully relieved through surgical management.

5448 - A464
Assessment of Corneal Endothelial Cell Loss With Pre and Postoperative Parameters in Cataract Surgery
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Purpose: The aim of this study is to assess pre and postoperative parameters respectively roll on corneal endothelial cell loss (ECL).

Methods: All patient presenting for cataract surgery from January to April 2009 were included. They were operated with same method, 3.2mm incision with divide and conquer technique. Everyone benefited cefuroxime intracameral injection. Age, sex, side, anesthesia mode, keratometry (Km), anterior chamber depth (ACD), axial length (AL). At 1 and 6 months were noted best corrected visual acuity (BCVA), central corneal thickness (CCT), endothelial cell density (ECD), Surgery time (T), absolute ultrasound time (UT), balanced salt solution volume (BSS). The difference between pre and postoperative parameters was analyzed with SPSS15.0 software.

Results: 90 patients were included, 63% female, 45 each side. Mean age is 72.8 years. Mean ECD in pre and postoperative were 2142±549 (MI) and 1969±549 (M6). ECD were 12.4%<0.01 and 11.03%<0.01 (M6), mean CCT were 231±56, 524±41 (MI) and 511±93 (M6), mean T was 16min±5, mean US was15sec±13, mean BSS was324±96, pre and postoperative BCVA average were 0.1±0.23 and 0.81±0.23, mean ACC and AL were 3.03±0.37 and 24.54±1.88, mean keratometry was 43.60±1.42. We found a significant statistic relation between ECL and age (p=0.034) preoperative ECD (p=0.002), ACC (p=0.019), US (p=0.001) and T (<0.012). It was existing a correlation between ACD and AL (p<0.001), Km and AL (p=0.003), US and T (p=0.001), US and BSS (p=0.001) and T and BSS (p=0.001).

Conclusions: The 11% ECL is conform to publication. Cefuroxime doesn’t seem to increase ECL. An endothelial cell fragility time-acquired explain the results. Others factors were already known on published study (T, US). Corneal endothelium needs to be preserved by optimizing operative parameters. Cefuroxime use doesn’t seem deleterious for endothelial cells.

CR: R. Coste, None; D. Denis, None.
Support: None

5445 - 5448
5450 - A466
A Rabbit Model for Teaching the Continuous Curvilinear Capsulorhexis

Purpose: To establish a realistic and practical model for teaching the continuous curvilinear capsulorhexis (CCC) in cataract surgery.

Methods: Whole-encuclated rabbit eyes were subjected to intracocular perfusion using one of three fixative treatment conditions, 2% paraformaldehyde (PFA) for 6 hours, 4% PFA for 30 minutes, and 4% PFA for 1 hour. Separately, explanted rabbit lenses were extracted and subjected to immersion in the same treatment conditions. Custom rabbit eye and lens holders were used to secure the preparations. Five cataract surgeons each with a minimum of 5 years experience performed a CCC on treated eyes and lenses per their usual fashion. The treatment conditions for the various specimens were masked, and the surgeons rated the lens capsule biomechanical properties encountered in each specimen in terms of similarity to that encountered in infants, children, adults, or the elderly. Surgeons also commented on how the performance of the CCC in ex vivo, treated or encuclated rabbit eyes compared to a CCC performed in human eyes in vivo.

Results: Explanted lenses treated with the 2% PFA for 6 hours, 4% PFA for 30 minutes, and 4% PFA for 1 hour were judged to resemble performance of a CCC on patients roughly 20, 70, and 50 years of age, respectively. Both explanted lenses and encuclated eyes were judged as providing realistic capsule biomechanical properties, with encuclated eyes additionally providing anterior chamber dimensions resembling those of the human eye. However, encuclated eyes exhibited variably dilated pupils and corneal clarity.

Conclusions: The majority of cataract surgery teaching models described in the literature focus on lens removal. Few have been developed explicitly for teaching the CCC, and none have involved model assessment by a panel of experienced surgeons. The model described is inexpensive, easy to prepare, and mimics the range of elasticity encountered in various patient populations, including the elderly human. As such it provides a realistic CCC practice model for the beginning cataract surgeon and may also have utility in teaching pupil expansion techniques.

CR: J.P. Ruggiero, None; C.G. Keller, None; A. Naseri, None; D.W. Sretavan, None.
Support: None
To evaluate the comfort and tolerance of topical anaesthesia during clear cornea phacoemulsification in previously vitrectomized patients.

**Purpose:** To evaluate the comfort and tolerance of topical anaesthesia during clear cornea phacoemulsification in previously vitrectomized patients.

**Methods:** Clear cornea phacoemulsification was performed under topical anaesthesia with amethocaine 0.5% without sedation in 25 eyes of 24 previously vitrectomized patients. The surgery was performed by a single, experienced surgeon. Patients were asked to rate the pain experienced during surgery using a 5-point visual analog scale, and to state if they would repeat the same kind of anaesthesia if they were to be operated on the fellow eye.

**Results:** Mean reported pain score was 1.28 ± 0.73 (range, 0 to 3). Only one patient reported a pain score of 3, and she also was the only one that would not repeat the same kind of surgery. Two eyes experienced posterior capsule rupture, and one eye presented anterior capsule dehiscence that could be solved without additional anaesthesia. The appearance of intraoperative complications did not influence the reported subjective pain.

**Conclusions:** Topical anaesthesia with amethocaine 0.5% without sedation provided excellent pain tolerance during clear cornea phacoemulsification in most of the previously vitrectomized patients for different causes.

CR: O. Golan, None; Y. Hod, None; O. Geyer, None.

Support: None

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**5455 - A471**

**Pupil Dilation With Intracameral Lidocaine During Phacoemulsification**

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**Purpose:** Topical mydriatic agents are usually used for pupillary dilation during cataract surgery but they may be associated with untoward side-effects. Intracameral lidocaine was reported to be a viable alternative. Pupil dilation by either topical mydriatics or intracameral lidocaine had been compared between patients’ eyes, with one receiving drops and the other receiving lidocaine. We now compared mydriasis by both methods in the same eye.

**Methods:** The pupils of 49 patients scheduled for unilateral phacoemulsification were dilated with topical cyclopentolate 1% and phenylephrine 10% during the preoperative visit and by intracameral preservative-free lidocaine 1% (0.2 mL) injected just before the procedure began. The horizontal pupil diameter was measured before and after pupil dilation using a caliper under operating microscope. Epinephrine (0.5 cc of 1:10000) was added to the irrigation fluid during surgery.

**Results:** The mean pupil dilation was 5.7 +/- 0.6mm with topical mydriatics and 4.1 +/- 0.8mm with intracameral injection (P < .0001). No statistically significant differences in abserved in male or female. patients with/without diabetes, eyes with light/darker colored iris, eyes with/without pseudoxefoliation. After intracameral injection adequate mydriasis (mean pupil size 6 +/- 0.8 mm) occurred within 90 seconds and pupil dilation was maintained or increased during the procedure. The overall subjective surgical performance was excellent. No patient required an intracameral mydriatic injection during surgery.

**Conclusion:** Greater pupillary dilation was achieved with topical mydriatics than with intracameral lidocaine. However, intracameral lidocaine provided adequate intraoperative pupil dilation in routine phacoemulsification surgery.

CR: O. Golan, None; Y. Hod, None; O. Geyer, None.

Support: None

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**5456 - A472**

**Validation of Najjar-Awwad Cataract Risk Score for Residents**

P.H. Blomquist, H.H. Winslou, J.W. Sargent. Ophthalmology, Univ of TX Southwestern Med Ctr, Dallas, TX.

**Purpose:** To validate the Najjar-Awwad cataract risk score for residents and the recommendation that beginning surgeons start with cases with risk scores <5.

**Methods:** Phacoemulsification cataract surgeries performed by 33 residents between April 2005 and December 2008 at 2 urban public county hospitals, Parkland Memorial Hospital (Dallas county) and John Peter Smith Hospital (Tarrant county), were retrospectively reviewed. Traumatic, congenital, and polar cataracts as well as lenses with dislocation or phacodonesis were excluded. Cases with incomplete documentation of risk factors were also excluded. The cataract risk score, which can range from 2-25, was calculated retrospectively for 1833 eligible cases. Intraoperative complications included posterior and anterior capsular tears, vitreous prolapse, dropped nucleus, and conversion to manual extracapsular cataract extraction. In addition to odds ratios, P values were calculated using chi square.

**Results:** There were 119 complications (6.5%), though the rate of complications involving vitreous prolapse or loss (including dropped nucleus) was only 3.2%. The significant risk factors included in the risk score associated with intraoperative complications were poor red reflex (odds ratio 2.1; 95% CI 1.4-3.1; P=0.00007) and dense nuclear sclerosis (OR 2.1; 95% CI 1.3-3.3; P=0.0004). Cataract risk scores ranged from 3-16. Only 85 cases (4.4%) had a score <5; while 359 (19.6%) had a score <6 and 885 (48.2%) had a score <7. The odds ratio was 1.1 (95% CI 1.04-2.8; P=0.80) for complications for score <5, 1.2 (95% CI 0.7-1.9; P=0.55) for score <6, and 2.1 (95% CI 1.4-3.1; P=0.0002) for score <7.

**Conclusions:** While the Najjar-Awwad cataract surgery risk score can be used to predict intraoperative complications at the time of cataract surgery, the complication rate did not significantly increase until the score reached 7. There was a paucity of cases with scores <5 in our county hospital populations. We recommend that beginning surgeons be given cases with risk scores at least <7.

CR: P.H. Blomquist, None; H.H. Winslou, None; J.W. Sargent, None.

Support: Supported in part by an unrestricted research grant from Research to Prevent Blindness, Inc., New York, New York, U.S.A.
Creation of Continuous Curvilinear Capsulorrhesis Using the iFS Femtosecond Laser in a Pig Eye Model


**Purpose:** The purpose of this study is to successfully use the iFS 60 kHz Femtosecond laser to create a consistent anterior curvilinear capsulorrhesis in the Pig eye model.

**Methods:** After being sacrificed for another study, 14 eyes were removed from 7 pigs at the time of their death. They were immediately put on ice. Three hours after sacrifice, eyes were taken to the Refractive Surgery center at Lackland AFB. Prior to laser treatment, anterior chambers were flattened by aspiration of aqueous with 25 gauge needle. The iFS laser 120kHz was set on IEK mode. A posterior side cut was set with an anterior depth of 300um and a posterior depth of 1200um. The diameter of the cut was set for 500um. After being docked on the laser, corneas were further compressed to allow laser to reach intended treatment zone. After laser treatment, each eye was immediately placed in formalin. For each eye, pathology slides were obtained. Three levels were taken with 20-30 microns between each level. Tissue stained with H & E, PAS, and one level left unstained.

**Results:** 13 eyes underwent laser treatment. One eye was discarded due to trauma to the eye while being removed from pig and was thus not treated. We were successfully able to create capsulorrhesis in many of the eyes. Specific data on number of eyes with successfully created capsulorrhesis and those with collateral damage are currently under investigation. Information on collateral damage to surrounding tissue, ie cornea, iris, lens will be reported.

**Conclusions:** The importance and relevance of being able to create a consistent 5.0mm capsulorrhesis would be very useful for improving cataract surgery. This is particularly true in complicated surgeries such as those with poor zonular support, pediatric cataracts (creating anterior or posterior capsulorrhesis), patients with small pupils, centration for premium lenses. In our study we used a wide range in our side cut (1200-300 um) ≈ 700 um because this was our first time attempting this procedure with the iFS and wanted to maximize our chance of catching anterior capsule. Future studies will attempt to minimize destruction to surrounding tissues and better isolate the anterior capsule. This data will help us tailor our settings in future attempts.

CR: M. Parsons. None.

Support: None
Methylglyoxal Increases the Solubility and Decreases the Aggregation Propensity of a Cataract Causing αA-Crystallin (AG98R) Mutant Protein

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Purpose: The AG98R mutation in human α-crystallin (αAG98R) causes cataract. The mutant protein exhibits increased oligomeric mass, decreased stability and altered chaperone function. The objective of this study was to determine effects of methylglyoxal (MGO), a metabolic dicarbonyl compound on the solubility and aggregation properties of αAG98R protein.

Methods: Wild-type α-crystallin and AG98R mutant proteins were expressed in E. Coli BL21 DE3 cells and purified by anion exchange chromatography. The purified proteins (0.2 mg) were incubated with (50 μM) or without MGO in 0.2 ml of 0.05 M sodium phosphate buffer (pH 7.2) at 37°C for one week. After incubation, the samples were filtered through 0.2 μm filter and 25 μl of the filtrate was injected to HPLC fitted with TSK500PW 

αΑ

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Conclusion: Our data suggests that MGO treatment increases the stability and decreases aggregation of αAG98R protein and thus MGO may have therapeutic role in preventing protein aggregation diseases.

CR: P. Santhoshkumar, None; M. Raju, None; K. Sharma, None.

Support: NIH Grant EY11981 and Research to Prevent Blindness

A Single Destabilizing Mutation (9%) Promotes Conected Unfolding of an Entire Globular Domain in ɣ-Crystallin

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Purpose: Conformational change and aggregation of native proteins is associated with many serious age-related and neurological diseases. ɣ-crystallin is a highly stable, abundant structural component of the vertebrate eye lens. A single 9% mutation in the N-terminal domain of mouse ɣ-crystallin causes the severe Opj cataract, with disruption of cellular organization and the appearance of fibrillar structures in the lens.

Methods: Multi-dimensional nuclear magnetic resonance (NMR) and several biochemical methods such as atomic force microscopy (AFM) were employed to study the destabilization effect of mutation.

Results: Although at room temperature the mutant protein has a near native fold, significant increases in hydrogen/deuterium exchange rates were observed by NMR for all the well-protected β-sheet core residues throughout the entire N-terminal domain of the mutant protein, resulting in up to 3.5 kcal/mol reduction in free energy of the folding/unfolding equilibrium. No difference was detected for the C-terminal domain. At higher temperature, this effect further increases to allow much more uniform exchange rate among the N-terminal core residues and those of the least well-structured surface loops. This suggests a concerted unfolding intermediate of the N-terminal domain while the C-terminal domain stays intact. Increasing concentrations of guanidinium HCl produced two transitions for the Opj mutant, with an unfolding intermediate at ~1M GuHCl. The consequence of this partial unfolding, whether by elevated temperature or denaturant, is the formation of Thioflavine T staining aggregates, which demonstrated fibril-like morphology by AFM. Seeding with already unfolded protein enhanced formation of amyloid-like fibrils. Congo red staining of Opj lens shows fairly large clumps of intensely fluorescent material in cortical fibers, reminiscent of the fibrils in the Opj cataractous lenses by EM images.

Conclusions: The Opj mutant protein provides a model for stress-related unfolding of an essentially normally folded protein and production of amyloid-like fibrillar aggregates.

CR: Z. Wu, None; G. Wistupa; S. Lee, None; B. Mahler, None; K. Wyatt, None; L. Dong, None.

Support: R21EY018423

Deamidation Induces Local Structural Changes in BetaB2 and BetaA3-Crystallins That Disrupt Stabilizing Interactions With Other Crystallins

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Purpose: To determine how deamidation-induced changes in betaB2-crystallin structure alter interactions among crystallins and with the chaperone, alphaA-crystallin.

Methods: Deamidation was mimicked by replacing glutamines with glutamic acids located at the monomer-monomer interface of the betaB2 and betaA3-crystallin homodimers. Deamidation-induced local structural changes were identified using hydrogen/deuterium exchange with electrospray ionization source mass spectrometry (HDMS). These changes were then correlated to stability during thermal unfolding and aggregation detected by dynamic light scattering. The chaperone requirement of deamidated betaB2-crystallin was determined using alphaA-crystallin. The protective interaction of betaB2 with betaA3 was also determined.

Results: HDMS revealed differences in deuterium incorporation between regions of wild type βB2-crystallin (WT), suggesting that the N-terminal domain (N-td) of βB2 was more accessible in solution than the C-terminal domain (C-td). Introducing glutamates at the interface decreased deuterium incorporation in the N-td and increased it in the C-td, lowering the temperature for unfolding and aggregation, which was rapidly followed by precipitation. This deamidation-induced aggregation and precipitation was completely prevented by alphaA-crystallin chaperone. Deamidation at the interface in betaA3 disrupted the linker peptide between the domains and altered the interaction sites with betaB1-crystallin.

Conclusions: Local structural changes due to deamidation were identified. These structural changes led to decreased stability and to altered interactions with other crystallins, including the protective chaperone, alphaA-crystallin. A potential mechanism for cataract formation in vivo may involve accumulation of deamidated betaB2-crystallin aggregates partially due to disruption of the stabilizing crystallin-crystallin interactions.

CR: K. J. Lampi, None; M. Michiel, None; T. Takata, None; E. Duprat, None; F. Skouri-Panet, None; S. Finet, None; A. Tardieu, None.

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Recognition by AlphaB-Crystallin Chaperone of C-Terminal Binding Regions in Human GammaD-Crystallin Substrates


Purpose: To identify the sites in partially folded human ɣD-crystallin recognized by the αB-crystallin chaperone during suppression of aggregation.

Methods: Human ɣB-crystallin and ɣD-crystallin were expressed in, and purified from bacteria. Human α-crystallin, one of the ubiquitous crystallins in vertebrate lenses, is composed of two polypeptide chains, ßA2 and ßB2, consisting of ~80 kD each, which are linked by a disulfide bond. Deamidation-induced aggregation and precipitation was not completely prevented by alphaA-crystallin chaperone.

Results: Human ɣD-crystallins refold through the population of a partially folded intermediate in vitro, which has its N-terminal domain unfolded and its C-terminal domain folded. At high protein concentrations aggregation of this species competes with productive refolding. Our previous work showed that the ɣD-crystallin substrate in ßD-cubes resembles this populated folding intermediate, with its N-terminal domain unfolded and its C-terminal domain folded. Using single domain constructs of ßD-crystallin, we have found that the partially folded C-terminal domain construct refolds, while the N-terminal domain construct (D-N) does not aggregate under similar conditions. Using chaperone constructs lacking tryptophans, we determined the fluorescence properties of the bound C-terminal domain, and found it corresponded to a partially folded chain. This complex was stable upon storage.

Conclusions: HoloCys can suppress the aggregation of partially folded species of the ßD-Cyd and forms stable ßD-Cyd-αB complexes. The results suggest that exposure of buried strands of the C-terminal Greek key are the determinants of ã-crystallin recognition and strong binding. The stability of the substrate/chaperone complex would result in saturation of the chaperone population in the lens nucleus, which may help explain the age dependence of mature-onset cataracts.

CR: J. A. King, None; I. Mills-Henry, None; L. Acosta-Sampson, None.

Support: NIH Grants EY013934 and GM17980
Organizing Section: LE

Earlier we reported identification of \( \beta \)-amyloid (-A\( \beta \)) and \( \alpha \)-crystallin from Neuroptix Corp., C; Ophthalmological examinations were correlated with phenotypic, histochemical, and \( \gamma \)-crystallin accumulation, cytosolic \( \beta \)-crystallin accumulation, co-localizing amyloid pathology, and disease-linked supranuclear cataracts in the lenses of subjects with DS. Here, we investigate the hypothesis that related AD-associated \( \alpha \)-crystallin lens pathology is expressed as an age-dependent early-onset lens phenotype in DS.

Methods: Ophthalmological examinations were correlated with phenotypic, histochemical, and biochemical analyses of lenses from subjects with DS, AD, and age-matched normal controls.

Results: Lenses from subjects with DS demonstrated lenticular A\( \beta \) cleavage peptides, accelerated cerebral A\( \beta \) accumulation, and invariant early-onset age-dependent AD neuropathology. The DS phenotype complex also includes high-penetration expression of distinctive early-onset cerebellar cataracts of unknown etiology. We previously reported pathogenic A\( \beta \) accumulation, co-localizing amyloid pathology, and disease-linked supranuclear cataracts in the lenses of subjects with AD. Here, we investigate the hypothesis that related AD-associated A\( \beta \)-crystallin lens pathology is expressed as an age-dependent early-onset lens phenotype in DS.

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6356 - 1:15PM
Characterization of the Porcine Eye-Lens Cortical and Nuclear Fiber-Cell Plasma Membranes
M. Raguz1, W.J. O'Brien2, W.K. Subczynski3, 1Biophysics, 2Ophthalmology and Microbiology, Medical College of Wisconsin, Milwaukee, WI.

Purpose: One of the most unique features of the eye lens fiber-cell plasma membrane is its extremely high cholesterol content. Cholesterol not only saturates the bulk phospholipid bilayer, but also induces formation of the immiscible cholesterol crystalline domains (CCD). The fiber-cell membranes are dense with membrane proteins. It has been estimated that more than 50% of lipid molecules in native lens membranes are in contact with intrinsic proteins. We hypothesize that the association of these proteins with membrane lipids should significantly affect the physical properties of the lipid-bilayer portion of the membrane.

Methods: Lipid n-doxylstearic acid spin labels and conventional as well as saturation recovery electron paramagnetic resonance (EPR) spectroscopy were used to assess physical properties of the lipid-bilayer portion of the plasma membranes including profiles of the order parameter, hydrophobicity, and the oxygen diffusion-concentration product (oxygen transport parameter).

Results: The physical properties, obtained for membranes containing membrane proteins, were compared with the properties of membranes made from the total lipid extract of porcine eye lenses, which did not contain proteins. We found that the lipid-bilayer portion of the intact membranes was significantly less fluid and better ordered than the lipid bilayer made of extracted lipids. Additionally, the movement of small hydrophobic molecules, such as molecular oxygen, was drastically restricted. However, hydrophobicity profiles obtained for biological and model membranes were nearly the same, showing the existence of a high hydrophobic barrier to movement of polar molecules across the fiber-cell plasma membrane. It was also evident that the rigidity of nuclear fiber-cell plasma membranes was greater than that of cortical membranes. The permeability of oxygen across the lipid portion of the fiber-cell plasma membrane (4.4 - 20.6 cm s⁻¹) was significantly lower than that across the lipid bilayer made of the lipid extract (57.2 - 65.1 cm s⁻¹).

Conclusions: The fiber-cell plasma membrane forms a significant barrier to oxygen transport, which should help maintain a low oxygen concentration in the eye-lens interior. A great deal of evidence suggests that an increase in oxygen concentration in the lens can lead to the development of cataracts. Our data provide the basis for a mechanism by which the transport of oxygen within the eye lens is controlled.

CR: M. Raguz, None; W.J. O'Brien, None; W.K. Subczynski, None.
Support: NIH Grants EY105526, TW2008052, and EY19031

6357 - 1:30PM
Effects of Hyaluronic Acid on Lens Epithelial Cell Migration and Proliferation
H.L. Chandler1, D.J. Haussler, Jr.1, E.A. Lutz1, D.A. Wilkie2, A.G. Metzler3, 1Optometry, 2Veterinary Medicine, The Ohio State University, Columbus, OH.

Purpose: To ascertain if canine lens epithelial cells (LEC) contain the hyaluronic acid (HA) receptors CD44 and CD168, and if surgical viscoelastics with higher HA content can negatively impact posterior capsular opacification (PCO) formation in vitro.

Methods: Canine LEC from normal (n=12) and cataractous (n=12) lenses were evaluated using immunohistochemistry and RT-PCR for expression of CD44 and CD168. Two in vitro models of PCO formation were used to determine if various HA concentrations altered LEC migration and proliferation. In the first model, a one millimeter scratch was created in cultures of confluent canine LEC. Cells were treated with 0, 0.2, or 1.0 mg/mL of HA dissolved in unsupplemented DMEM. Migration of LEC into the scratch was monitored for 24 hours and quantitated using ImageJ. In the second model, mock cataract surgery was performed on canine cadaver eyes. Following removal of lens fibres, capsules were re-distended using one of the following treatments (n=6): no viscoelastic (DMEM only), 0% HA viscoelastic, 1% HA viscoelastic, or 2.0% HA viscoelastic. The treatment remained in the lens capsule for 5 minutes before removal by irrigation and aspiration. Capsules were monitored daily and the rate of migration and proliferation onto the posterior capsule was evaluated.

Results: Both normal and cataractous LEC were positive for CD44 and CD168 protein and mRNA. There was a significant increase in the rate of migration in LEC treated with 0.2 and 1.0 mg/mL of HA compared to LEC receiving 0 mg/mL of HA (p=0.001). LEC treated with either 1.2% or 2.0% HA viscoelastics reached confluence on the posterior capsule significantly faster than LEC receiving no or 0% HA viscoelastic (p=0.003).

Conclusions: Canine LEC possess the appropriate receptors to respond to HA signaling; however, the positive effects of HA on cell migration and proliferation were not observed in vitro.

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Support: Aircrivet, Inc; ACVO Vision for Animals Foundation

6358 - 1:45PM
Enhancement of Ubiquitin-Proteasome Pathway (UPP) Reduces Aggregation of Mutant Crystallins and Provides Cytoprotection
F. Shang1, Q. Biao1, A. Taylor1, M. Wu2, JJ. Liang1. 1Human Nutrition Res Ctr on Aging, Tufts University, Boston, MA; 2Zhongshan Ophthalmic Center, Sun Yat-Sen University, Guangzhou, China; 1Center for Ophthalmic Research, Brigham and Women’s Hospital, Harvard University, Boston, MA.

Purpose: Accumulation and precipitation of abnormal proteins are associated with many age-related diseases, including cataract. To maintain cell function and protein homeostasis, organisms have evolved protein quality control mechanisms, which include the UPP to selectively degrade damaged proteins, and molecular chaperones to refold misfolded proteins. The objective of this study is to determine the interaction between the UPP and molecular chaperones and to test the effects of enhancement of the UPP on aggregation of mutant crystallins and cytoprotection against environmental stress.

Methods: To enhance the UPP function we overexpressed a rate-limiting ubiquitin ligase (CHIP) or CHIP together with its functional partner, Ubc5. CHIP or CHIP together with Ubc5 were expressed in confluent human lens epithelial cells. The levels of CHIP, Ubc5, ubiquitin conjugates and molecular chaperones were determined by Western blot. mRNA levels of molecular chaperones were determined by Real-Time RT-PCR. RFP-tagged WT and R120Gmutant α-crystallins were co-expressed with CHIP or CHIP and Ubc5 in HeLa cells. Aggregates of α-crystallins in HeLa cells were detected using confocal microscopy.

Cell viability was determined by MTS assay.

Results: Over-expression of CHIP in HEKC increased the levels of ubiquitin conjugates and many cytoplasmic chaperones, but not ER chaperones. Among the cytoplasmic chaperones, mRNA level of αB-crystallin, Hsp70 and Hsp27 increased 3-fold, 4-fold and 2-fold respectively. Over-expression of CHIP in HEKC significantly reduced the cytotoxicity caused by L-carnitine, an amino acid analog which results in abnormal proteins. Over-expression of CHIP together with CHIP confers stronger protective effects against L-carnitine than induction of CHIP alone. Furthermore, expression of CHIP and Ubc5 in HeLa cells reduced the number and size of perinuclear aggregates of R120G mutant α-crystallin.

Conclusions: These data show that the UPP plays an important role in coping with abnormal proteins. Enhancement of functions of the UPP via over-expression of CHIP or CHIP and Ubc5 may be an effective therapeutic approach to prevent protein aggregation and protein aggregation-associated diseases, such as cataract.

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Support: NIH EY011717 (to FS) and EY013250 (to AT); USDA CRIS 1950-51000-060-02A

6359 - 2:00PM
The Function of β1 Integrin on the Lateral Membranes of Lens Fiber Cells
D.A. Scheiblin1, V.N. Smirskii, K.J. Czymmek, M.K. Duncan. Biological Sciences, University of Delaware, Newark, DE.

Purpose: Previous work has shown that the lack of β1 integrin in the embryonic lens causes an abnormal mesenchymal transition of the lens epithelium followed by apoptosis. While lens fiber cells are also defective, it is unknown whether these defects arise secondarily due to the loss of the epithelial cells or reflect an important primary role for β1 integrin in the fibers. This work investigates the role of β1 integrin in lens fiber cells.

Methods: The conditional deletion of β1 integrin in lens fiber cells had variable phenotypes ranging from severe cataract to a grossly transparent, clear lens. Irrespective of gross phenotype, the vertices of β1 integrin and C57Bl/6 wildtype controls were assessed by darkfield microscopy. Lens ultrastructure was assessed using scanning electron microscopy (SEM).

Results: The conditional deletion of β1 integrin resulted in the removal of exon 3 of the gene causing premature translational termination at amino acid 37. Mice lacking β1 integrin in lens fiber cells had variable phenotypes ranging from severe cataract to a grossly transparent, clear lens. Irrespective of gross phenotype, the vertices of the cortical fiber cell lateral membranes lacking β1 integrin had disordered ball and socket structures instead of the highly regular structural repeats of wildtype lens fiber cells. This became progressively more severe in older cortical fibers, which exhibit ball and sockets of variable width and extension from the cell. Finally, nuclear fibers lacking β1 integrin completely lack ball and sockets and instead have smooth lateral membranes. In wildtype lenses, membrane furrows were found in a random pattern on the lateral sidechylar, Hair fibers. In contrast, the membrane furrows in nuclear fibers lacking β1 integrin were of higher density, had more pronounced ridges and were highly organized into a lattice-like geometric pattern.

Conclusion: The function of β1 integrin protein is required to form the appropriate morphology of lens fiber cell lateral membranes.
6360 - 2:15PM
**PEDF RNAi Altered Vimentin and αB-Crystallin Expression in Human Lens Epithelium**

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**Purpose:** To study relationship of Pigment epithelium-derived factor (PEDF) expression with the expression of vimentin and αB-crystallin by lens epithelial cells.

**Methods:** Lens epithelial cells adhering to anterior capsules taken from young donor eyes aged from 20-35 years were cultured and passaged. We designed siRNA constructs to specifically down regulate the expression of Pigment epithelium-derived factor (PEDF) by those primary lens epithelial cells. Quantitative PCR was used to confirm the down regulation of PEDF RNA expression following infection of lens epithelial cells. In order to determine whether altering the expression of PEDF would effect the expression of vimentin or αB-crystallin, we performed western blotting 48 hours after expression of the PEDF directed siRNA.

**Results:** PEDF RNA expression in the human lens epithelial cells was strongly down regulated by the three separate siRNA constructs. Western blotting revealed that the down regulation of PEDF expression resulted in a concomitant decrease in expression of vimentin and an increase in αB-crystallin expression.

**Conclusions:** Decreased expression of PEDF by primary human lens epithelial cells results in a decrease in the expression of vimentin and the increase of αB-crystallin expression, two proteins critical for maintaining lens clarity.

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Support: CNSF grant (30772391)

6360 - 2:15PM
**Alpha-Crystallins Interact With Caspase-3 and Bax in vitro and in vivo to Prevent Stress-Induced Apoptosis and Cataractogenesis**

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**Purpose:** To study how alpha-crystallins regulate stress-induced apoptosis.

**Methods:** The interaction between alpha-crystallins and caspase-3 as well as Bax were detected using in vitro and in vivo approaches. Alpha-crystallins directly interact with caspase-3 as well as Bax to prevent stress-induced apoptosis.

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6361 - 2:30PM
**A Molecular Dynamics Study of the AQP0-CaM Interaction**

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**Purpose:** Aquaporin 0 (AQP0) is a water transport channel comprised of more than 60% of the protein in lens cell membranes. The water permeability of AQP0 is regulated by intracellular calcium levels through calmodulin (CaM) binding. Altered sensitivity to calcium levels in response to phosphorylation of the C-terminal α-helix has also been observed. The purpose of this study is to elucidate the molecular mechanisms by which AQP0 is regulated by CaM. Specifically, we use atomistic molecular dynamic simulations to explore how various states of phosphorylation influence the interaction between the C-terminal domains of AQP0 in the native (tetrameric, membrane embedded) form with CaM.

**Methods:** We ran molecular dynamics simulations with a starting structure adapted from a model proposed by Reichow and Gonseth (Structure 2008).

**Results:** Our simulations show that CaM can interact with the C-terminus of AQP0 stably in more than one conformation, indicating that there may be various conformational states of the AQP0-CaM complex resulting from different physiological conditions. Furthermore, we observe lower water occupancy of the pores of the AQP0 tetramer in the CaM bound state, showing both that the binding of CaM can block water transport by AQP0 and that the mechanism of transport blockade is occlusion of the pores rather than conformational change of the pore itself.

**Conclusions:** Our molecular dynamics simulations of the AQP0-CaM complex confirm previous hypotheses that AQP0 is regulated by calcium through the occlusion of the transmembrane pores of the AQP0 tetramer by CaM. Additionally, our simulations give insight to the molecular mechanisms behind the altered calcium sensitivity of AQP0 in various states of C-terminal helix phosphorylation.

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