2884 - B437

Synthesis of Kynurenic Acid, a Tryptophan Derivative in Cataractous Lenses

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Purpose: Kynurenines and their glycoside derivatives absorb UV radiation, thus, they may be useful in protecting the retina from UV light. Kynurenine aminotransferases (KAT I and II) are pivotal for the synthesis of kynurenic acid (KYNA). The experiments were designed to investigate KYNA synthesis in human cataractous lenses and in experimental cataract in diabetic rats.

Methods: KYNA levels were investigated with HPLC and detected fluorimetrically. Nuclei of human cataractous lenses collected following planned extracapsular extraction were classified accordingly to LOCS III and compared to clear lenses with regard to KYNA concentration. Cataractous lenses from STZ-treated rats were compared to controls. Immunohistochemistry was performed using polyclonal antibodies against KAT I or KAT II on human lenses.

Results: In clear lenses (NC0), KYNA concentration was 0.95 ± 0.22, in NC1 was 0.8 ± 0.72, in NC2 was 1.18 ± 0.88, in NC3 was 1,31 ± 0.70, in NC4 was 1.78 ± 0.92, in NC5 was 8.80 ± 8.28 ($P<0.05$ vs NC0) and in NC6 was 14.0 ± 11.1 ($P<0.05$ vs NC0). Correlation between the grade of cataract (LOCS III scale) and KYNA concentration was noted ($r=0.047$, $P<0.001$). Elevated KYNA concentration in rat cataractous lenses was observed ($P<0.05$). Up-regulation of KAT I and KAT II expression depending on development of cataract was also noted.

Conclusions: Elevation of KYNA levels and up-regulation of KAT I and II expression in cataractous lenses may suggest potential role of KYNA synthesis in mechanisms of cataract formation.

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2885 - B438

3-Hydroxykynurenine-Mediated Modification of Lens Proteins: Detection by a Monoclonal Antibody

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Purpose: Tryptophan undergoes oxidation by enzymatic and non-enzymatic mechanisms to produce kynurenines. Kynurenines are highly reactive molecules; they react with proteins induce crosslinking and generate yellow brown pigments in the lens. In this study we have investigated effects of glycation on tryptophan oxidation and the role of kynurenines in lens protein modification. **Methods:** A monoclonal antibody was generated against 3-hydroxykynurenine (3-OHK)-modified KLH. The antibody was characterized using 3-OHK-modified amino acids and proteins. Effect of early and advanced glycation products on kynurenine-modification was studied using glycated amino acids and glycated lysine-Sepharose. A major antigen for the antibody was purified from the reaction of 3-OHK and N^{α}-acetyl lysine by reversed phase HPLC. Immunohistochemistry, Western blotting and a competitive ELISA were used to detect or quantify 3-OHK-derived products in human lens proteins. 3-OHK-mediated modification was also investigated in human lens epithelial cells incubated in the presence of 3-OHK. **Results:** In competitive ELISAs, the monoclonal antibody reacted similarly with 3-OHK-modified N^{α}-acetyl lysine, N^{α}-acetyl histidine, N^{α}-acetyl arginine and N^{α}-acetyl cysteine. Addition of tryptophan during glycation or incubation of proteins with early glycation products did not generate kynurenine modifications on proteins. Among several tryptophan oxidation products tested, 3-OHK produced the highest antigen level when reacted with human lens proteins. A major antigen was isolated from the reaction of 3-OHK and N^{α}-acetyl lysine and was characterized by spectroscopy. Western blotting detected 3-OHK-modifications in enzyme digested proteins from cataractous but not non-catarctous lenses. Immunohistochemistry revealed 3-OHK modifications in proteins associated with the fiber cell plasma membrane in cataractous lenses. Low molecular products (<10,000 kDa) isolated from cataractous lenses after treatment with glucosidase and incubation with proteins generated 3-OHK-modifications. Human lens epithelial cells (HLE B-3) incubated with 3-OHK showed intense immunoreactivity. Western blotting revealed 3-OHK modifications in high molecular weight proteins of the cytosolic fraction. **Conclusions:** Tryptophan oxidation to kynurenines does not occur during glycation. 3-OHK-derived products crosslinked to basic amino acids and cysteine are likely to be present in cataractous lenses, which may contribute to protein aggregation and browning during cataract formation.

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2886 - B439

Presence of 3-Oxidopyridinium Derivatives of Lysine in Human Lens

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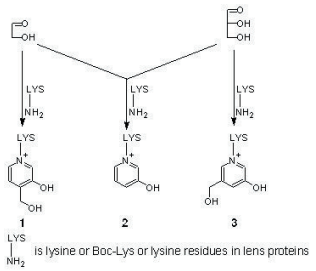
Purpose: In a previous study we reported that a compound having the structure **2** could be detected in digested human lenses using LS-MS/MS. We reported that the structure **2** could be formed by the combined action of glycolaldehyde and glyceraldehyde. Recently we found that structures **1** and **3** could be formed by the individual action of glycolaldehyde or glyceraldehyde on Boc-Lys in vitro. The purpose of this study was to find if structures **1** and **3** could be detected in digested human eye lenses.



Methods: Human lenses were subject of digestion using a set of proteolytic enzymes. ^{13}C labeled standards of compounds **1** and **3** were added to the digest obtained. HPLC fractions containing the standards were collected. MS and MS/MS spectra of the fractions corresponding to the standards were recorded.

Results: The MS and MS/MS spectra suggest that both the ^{13}C labeled standards and the corresponding non-labeled products were present in the fractions collected. This is an evidence that structures **1** and **3** exist in human lens.

Conclusions: The presence of structures **1**, **2** and **3** in lens proteins suggests that glycolaldehyde and glyceraldehyde are effective glycation agents in human eye lens according to the scheme given bellow.

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2887 - B440

Glycation-Induced Aggregation of Lens Proteins by the Oxidation Products of Ascorbic Acid

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Purpose: To determine whether the glycation of calf lens proteins in vitro by the oxidation products of ascorbic acid is able to cause protein aggregation.

Methods: Dialyzed water-soluble (WS) proteins from fetal calf lenses were incubated with 20 mM ascorbic acid under air for 4 weeks with aliquots removed at weekly intervals. Each aliquot was analyzed by Superose-6 gel filtration chromatography, monitoring for protein at A_{280nm} and for advanced glycation endproducts (AGEs) at A_{330nm}. Similar experiments were carried out with 1.0 mM ascorbate in the presence and absence of 100 mM cyanoborohydride, 2-aminoguanidine or semicarbizide, all of which inhibit AGE formation. The incorporation of ascorbate into aggregated proteins was also determined following the incubation of 10 mg/mL calf lens proteins with 20 mM[U-^{14}C]ascorbate (0.24 mCi/mmole). These data were compared to the Superose-6 chromatographic profile of the WS proteins from aged human lenses.

Results: The aggregation of calf lens proteins was observed after only one week of incubation with 20 mM ascorbate in air. Aggregate peaks eluted prior to α-crystallin (~1-2 x 10^6 Da), which may represent chaperone complexes, and in the void volume of the Superose-6 column as a high molecular weight (HMW) peak of 4 x 10^7 Da or larger. Both aggregate peaks increased proportionately over the next 3 weeks with high levels of yellow compounds associated with each peak. No aggregation or yellowing was seen in protein samples incubated without ascorbate. Incubations with 1.0 mM ascorbate also showed extensive protein aggregation. A similar HMW peak was seen when the WS proteins from aged human lens were separated. Glycation was implicated by the incorporation of radioactive ascorbate into each of the aggregate peaks. Further, the addition of cyanoborohydride or other inhibitors of AGE formation blocked protein aggregation. Absorption spectra of the glycated proteins showed a correlation between the extent of yellowing and aggregate formation.

Conclusions: Protein aggregation increases during nuclear cataract formation until the aggregate size is large enough to scatter light (cataract). Increasing evidence argues that AGE formation due to the oxidation products of ascorbic acid is a prominent feature in aged and cataractous lenses. We show here that ascorbate glycation alone can perturb the structure of fetal calf lens proteins sufficiently to cause them to aggregate into very high molecular weight complexes similar to those seen in aged human lenses.

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2888 - B441

Glycation of Ubiquitin May Underlie the Molecular Mechanism of Diabetic Cataract

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Purpose: The ubiquitin-proteasome pathway (UPP) plays critical roles in various cellular processes, such as protein quality control and stress responses. Glycation of cellular proteins by glucose or its metabolites may be a causal factor for many complications of diabetes. The objective of this study is to investigate the susceptibility of ubiquitin to glycation and to study the physiological consequences of glycation of ubiquitin in the lens or cultured human lens epithelial cells (HLEC).

Methods: Ubiquitin was incubated with glucose-6-phosphate or methyglyoxal. The sites and extent of glycation were determined by mass spectrometry-based peptide mapping. The effects of glycated ubiquitin on proteolysis were determined using αA-crystallin as a substrate in reticlolyte lysate . The effects of methyglyoxal on the UPP were also determined in cultured HLEC.

Results: Ubiquitin was readily glycated by glucose-6-phosphate and methyglyoxal. Whereas glucose-6-phosphate modifies ubiquitin primarily on Lys-11, methyglyoxal modifies ubiquitin mainly at Lys-48 and Arg-42. Both glucose-6-phosphate- and methyglyoxal-modified ubiquitin inhibit ATP-dependent degradation of αA-crystallin in reticulocyte lysates. Glycated ubiquitin was efficiently incorporated into poly-ubiquitin chains but the conjugates formed with glycated ubiquitin accumulated rather than being degraded by the 26S proteasome. Treatment of HLEC with methyglyoxal also resulted in accumulation of endogenous ubiquitin conjugates. Levels of ubiquitin conjugates in galactosemic cataract lenses were significant higher than those observed in control lenses. The accumulation of ubiquitin-conjugates was not due to inactivation of proteasome, because we found that the chymotrypsin-like peptidase activity of the proteasome was higher in methyglyoxal-treated HLEC or galactosemic cataract lenses.

Conclusions: Ubiquitin is susceptible to glycation by glucose or its metabolites. Glycation of ubiquitin impairs functions of the UPP and results in severe physiological consequences. Modifications of ubiquitin by glucose or its metabolites may underlie the molecular mechanism of diabetic cataract.

CR: X. Zhang, None; **C. Marques**, None; **M. Gallagher**, None; **A. Taylor**, None; **P. Pereira**, None; **F. Shang**, None.

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2889 - B442

Covalent Modifications of Glutathione by Glucose

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Purpose: We explored a possibility that glucose, which is present in diabetic lens in mM concentration could react non-enzymatically with reduced and oxidized forms of glutathione, resulting in the formation of the correspondent Amadori compounds.

Methods: Sterile solutions of reduced (GSH) and oxidized (GSSG) forms of glutathione were incubated with glucose in rations 1:5 through 1:200 in 50 mM phosphate buffer (pH 7.0), containing 1 mM DTPA under argon for the duration of 48 hours at 37°C in dark. Aliquots of these reaction mixtures were acidified and analyzed by a gradient RP-HPLC equipped with mass spectrometric detector. Normal human aged lenses (55-75 years old, 10 lenses) and cataractous lenses of Indian origin (55-75 years old, 10 lenses) were homogenized in de-gassed and ice-cold 10% TCA and centrifuged. After ether extraction of TCA from the lens supernatants, these low-molecular weight fractions were analyzed by RP-HPLC-MS for the presence of Amadori compounds of glutathione

Results: Under the chromatographic conditions used in these studies we observed time- and concentration-dependent formation of N-1-Deoxy-fructosyl GSH (FGH) as the major glycation product of GSH with glucose. FGH had a characteristic positively charged ion with m/z=470 Th in its LC-MS spectra. Mixtures of GSSG and glucose generated two compounds, N-1-Deoxy-fructosyl GSSG (FGG; m/z=775 Th) as major adduct and bis di-N, N'-1-Deoxy-fructosyl GSSG (FGGF; m/z=937 Th) as the minor one. All three compounds showed a resonance signal at 55.2 ppm in the ^{13}C-NMR spectra as C(1) methylene group of deoxyfructosyl, which represents a direct evidence that they are Amadori compound. All three compounds also showed LC-MS/MS fragmentation patterns identical to the synthetically synthesized FGH, FGG and FGGF. FGH was shown to be a poor substrate for the lenticular GPx (6.7% of the enzyme's original specific activity, SP), G-S-T (25.7% of the original enzyme's SP) and glyoxylase I (32% of the enzyme's SP). GR failed to recycle the disulfide bond within the structure of di-substituted FGGF, showing only 1% of the original enzyme's SP, but retained its ability to recycle FGG by 57% of its original SP. In addition, the presence of FGH was found in the low molecular fractions from aged human lenses and in higher amounts in brunescent cataractous lenses, as was demonstrated by LC-MS analysis.

Conclusions: The evidence of the presence of FGH in aged human normal and cataractous lenses argues that the reaction between GSH and glucose, which leads to the formation of FGH, can be responsible, at least in part, for the depletion of levels GSH in human lens with aging and in diabetic cataract development

CR: M.D. Linetsky, None; **E.V. Shipova**, None; **R.D. LeGrand**, None; **O.K. Argirov**, None.

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2890 - B443

Characterization of Lens Protein Modifications by Ascorbic Acid and Other Carbonyl Agents With 2-D LC-MS MAP

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Purpose: The glycation of calf lens proteins (CLP) by ascorbic acid produces yellow chromophores and fluorophores, which are chromatographically and chemically identical to those isolated from aged human and cataract lens proteins. In the work presented here our aim was to determine using 2-D LC-MS MAP analysis whether other glycating agents could also produce chromophores common to those in human lens. **Methods:** CLP (10 mg/ml) were incubated with either 200 mM glucose or 20 mM ascorbate, ribose, erythrulose or methyl glyoxal (MGO) at 37 °C in Chelex-treated phosphate buffer for 4 weeks. Each reaction mixture was dialyzed and the glycated proteins were digested with proteolytic enzymes. The AGE-containing peaks were isolated from a small Bio-Gel P-2 column and subjected to an in-line LC-mass spectrometer equipped with an electrospray interface. Several inhibitors were employed at a concentration of 10 - 50 mM to confirm that the peaks were due to glycation. All the LC-MS data were analyzed using 2-D MAP technique. **Results:** (a). Bio-Gel P-2 profiles were similar for all the glycated CLP digests, but the reactivity of the various glycating agents for the formation of yellow chromophores with lens proteins were: MGO>L-ascorbic acid>L-erythrulose>D-ribose>D-glucose. (b). Ascorbic acid and L-erythrulose, which is a breakdown product of oxidized ascorbic acid, produced identical modifications confirmed by LC-MS MAP analysis. Glucose, ribose and MGO generated several modifications same to ascorbate and erythrulose, but in much lower levels. (c). Aminoguanidine and cyanoborohydride inhibited almost 80% of the glycation reactions. **Conclusions:** The modifications formed from ascorbic acid and L-erythrulose was identical. The other carbonyl compounds generated several modifications same as ascorbic acid but in much lower concentrations, suggesting that the AGEs, which accumulate with age in human lens arise mainly from ascorbic acid oxidation products.

CR: R. Cheng, None; **B.J. Ortwerth**, None.

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2891 - B444

The Unfolded Protein Response (UPR) Induced by Endoplasmic Reticulum (ER) Stress; A Base for Cataract Formation in Ocular Lens

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Purpose: The pivotal role of ER in the life of cells has been known, but the part it plays in the progression of human diseases has only recently been appreciated. We hypothesize that unfolded protein aggregates are recognized in the endoplasmic reticulum (ER) in lens epithelial cells (LECs) and generate the unfolded protein response (UPR), which initiates diverse signaling responses to eliminate unfolded protein aggregates. In the UPR, GRP78/Bip releases 3 proteins : IRE1, ATF6 and PERK. PERK phosphorylates eIF2α, and eIF2α-P activates ATF4, CHOP and other chaperon proteins. However, if they fail to remove the aggregates, the cell undergoes apoptosis. Cataract is a major ocular disease and apoptotic LECs are considered to be an etiology. We investigated whether the UPR induced by ER stress leads to apoptosis in LECs. **Methods:** Human LECs were cultured under hypo-(0 mM) or hyper-(300 mM) glucose, three experimental UPR inducers [homocysteine (5 mM), Ca^{++} ionophore A23187 (1 μM), or tunicamycin (5 μg/ml)] and three environmental stress conditions [oxidative (10 μM of H_2O_2), heat(42°C), or osmotic(50 mM galactose)]. Total proteins were extracted from the cells. Protein blot analysis with antibody to UPR enzymes, GRP78/Bip, ATF4, CHOP, LEDGF and Caspase-12 was conducted to quantify the amount of each enzyme. **Results:** Elevated levels of UPR enzymes were found in the LECs treated with homocysteine, Ca^{++} ionophore A23187, tunicamycin and hypo-glucose for 24 hrs. These results indicated that the UPR was induced by ER stress in LECs. Levels of the UPR enzymes did not change in LECs treated with three environmental stresses and hyper glucose for 24 hrs, indicating that these stresses did not induce the UPR. **Conclusions:** The UPR was induced by homocysteine, Ca^{++} ionophore, and tunicamycin and deprivation of glucose in LECs. The UPR is one of the important apoptotic pathways in LECs and cataract formation. Recently, the UPR has been also known to generate reactive oxygen species (ROS) in cells, which induces the UPR dependent apoptosis. We will further discuss whether ROS generated by the UPR induce LEC death.

CR: K. Ikesugi, None; **M.L. Mulhern**, None; **R. Yamamoto**, None; **T. Shinohara**, None.

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2892 - B445

Modification of Cytoskeletal Proteins by Lipid-Derived Aldehydes in the Lens Epithelial Cells

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Purpose: We have demonstrated earlier that 4-hydroxynonenal (HNE), a reactive lipid derived aldehyde is cataractogenic . It has been shown to modify the neuronal microtubules and cytoskeleton of fibroblasts and cardiomyocytes . Because disrupted lens cytoskeleton in the animal models of cataract and human cataract have been observed, we investigated if HNE mediates the disruption of lens cytoskeleton under conditions of oxidative stress. **Methods:** Human lens epithelial cell line (HLEC) and rat lenses were exposed to HNE or oxidants such as Fenton reagents and 50 mM glucose. In some experiments butylated hydroxytoluene (BHT) was added to ameliorate the oxidative stress. Apoptosis of HLEC was measured by the cell death detection ELISA kit and the opacity of the rat lens was measured using the image system. Immunohistochemistry and Western blots of HLEC and rat lens epithelia were performed using cytoskeletal antibodies such as anti-tubulin, anti-actin and anti-vimentin. **Results:** HNE induced apoptosis in HLEC starting as early as 1 hr of exposure as compared to 4 hrs by Fenton reagents. Both, HNE and Fenton reagents produced opacification in the rat lens at 72-96 hrs of incubation. Immunohistochemical studies and Western blots demonstrated a disruption in the cytoskeleton as well as loss of cytoskeletal proteins. **Conclusions:** Under oxidative stress, modification of the cytoskeletal proteins by HNE could be an important event resulting in apoptosis of the lens epithelial cells and lens opacification. Scavenging the oxidants and detoxification of HNE , in combination, may be an efficient preventive approach against oxidation-induced cataractogenesis.

CR: M. Zhang, None; **T. Xiaio**, None; **L. Vergara**, None; **N. Ansari**, None.

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2893 - B446

Estrogen Localization and Local Synthesis of Estrogen by Cataractous LEC

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Purpose: Our laboratory has identified overexpression of estrogen receptor alpha (ERa) in cataractous LEC. We know that ERa can function in both an estrogen-dependent and -independent manner. Furthermore, sulfatase and aromatase enzyme activity are necessary for the synthesis of active estrogen (17 beta estradiol). We hypothesized that in order for ERa to function in an estrogen-dependent manner in cataractous LEC, estrogen would have to be not only present in the LEC or aqueous humor, but there had to be local production of 17 beta estradiol within the anterior segment of the eye. **Methods:** Samples included normal whole lenses collected from dogs euthanized at a local animal shelter for overpopulation reasons, anterior capsulotomy samples from dogs with naturally occurring cataracts, and aqueous humor collected from both populations prior to dissection or surgery, respectively. Immunohistochemical staining was performed using antibodies against 17 beta estradiol and sulfatase. In addition, real time RT-PCR was used to measure aromatase transcription, an accepted method to evaluate the final step in the synthesis of 17-beta estradiol. **Results:** Immunohistochemistry found that cataractous LEC had more intense immunostaining for 17 beta estradiol and sulfatase in the cytoplasm and nucleus than normal LEC, which had primarily cytoplasmic immunolocalization of both proteins. RT-PCR found significantly up-regulated aromatase transcription in cataractous LEC and only negligible levels in normal LEC. **Conclusions:** The synthesis of 17 beta estradiol requires the activity of at least one of 2 enzymes, sulfatase and aromatase. This is the first evidence of 17 beta estradiol production by cataractous LEC supported by the positive immunostaining for 17-beta estradiol and sulfatase, and up-regulated transcription of aromatase by RT-PCR. These findings, combined with overexpression of ERa in cataractous LEC, suggest that LEC may become estrogen-responsive during or due to cataractogenesis. Perhaps, the presence of estrogen in normal LEC, without local its production or ERa overexpression, is a protective mechanism against cataractogenesis, but the up-regulation of ERa and local production of estrogen result in cataractogenesis.

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2894 - B447

Overexpression of ER alpha in Canine Lens Epithelial Cells Results in Up-Regulation of TERT and TR

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Purpose: Estrogen has been shown to protect the lens from cataract formation via estrogen receptor alpha (ERa). ERa can bind the catalytic subunit of telomerase (TERT) promoter and up-regulate telomerase activity in estrogen dependent cells. Our laboratory has found that ERa and TERT interact in cataractous LEC but not in normal LEC. We intend to determine if ERa overexpression leads to increased transcription of the telomerase components, TERT and TR. **Methods:** Canine ERa was cloned by RT-PCR. The canine ERa gene was sub-cloned into the eukaryotic expression vector pIRES-hrGFP-2a for transfection experiments. Primary cultures of canine LEC were transfected with FuGene 6 Reagent and ERa/pIRES-hrGFP plasmid DNA for 48 hr according to the manufacturers protocol. Estrogen was added to the culture 16 hr before cells were harvested. RNAs extracted from lens tissues and LEC were used for the first strand cDNA synthesis. Relative quantitative real time RT-PCR was performed in the Mx3000p System (95C for 10 minutes, then 40 cycles of 94C for 30 seconds, 60C for 30 seconds, and 72C for 30 seconds) using QuantiTect SYBR Green PCR kit. The relative quantification of TR, TERT, and ERa, normalized to HPRT (housekeeping control), are calculated using the LinRegPCR software. The primers to amplify sequences of the TR, TERT, ERa, and HPRT genes were designed based on the sequence data obtained in our laboratory. **Results:** ERa, TR, and TERT expression levels are higher in cataractous LEC than in normal LEC by relative quantitative real time RT-PCR. Preliminary data found ERa to be up-regulated in the presence and absence of estrogen after overexpression. In addition, TERT and TR were up-regulated in an estrogen-dependent in response to ERa overexpression. **Conclusions:** This data supports that ERa expression regulates the transcription of TERT and TR in LEC in an estrogen dependent manner. The future experiments are planned to check whether the proteins, ERa, TERT, and TR, are also increased in ERa transfected LEC.

CR: P. Lu, None; **C.A. Barden**, None; **H.L. Chandler**, None; **Y. Sugimoto**, None; **C.M.H. Colitz**, None.

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2895 - B448

Ionomycin Causes Opacities in Cultured Sheep Lenses by Damaging the Outer Layers of the Cortex

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Purpose: Cultured lenses treated with the calcium ionophore, ionomycin, have been used as model systems to test the efficacy of potential therapeutic agents for cataracts. The initial purpose of this experiment was to determine which parts of the lens were affected by ionomycin. The second objective was to look for specific evidence of calpain activation during opacification. **Methods:** Lamb eyes were dissected and the lenses were cultured in Eagles minimum essential medium (EMEM) for 48 hr. Lens opacity was induced in the test group by exposure to 1 or 2μM ionomycin for up to 5 days. Control lenses either remained in EMEM or EMEM plus ionomycin and EGTA. Following culture some lenses were fixed and the cell structure determined. The remaining lenses were dissected into epithelium, cortex, and nucleus. The calcium concentration, calpain activity and extent of proteolysis were determined in each fraction. Proteolysis of lens proteins was assessed by 2-dimensional electrophoresis (2-DE) and by Western blotting of spectrin and vimentin. Spots from 2-DE gels were identified by mass spectrometry and by comparison with previously mapped lens proteins. **Results:** Control lenses cultured in EMEM remained transparent over the 5-day period. Ionomycin initially caused swelling of cells and opacification at the lens equator which became more generalized. Calcium chelation with EGTA reduced opacification. Calpain 2 activation in the epithelium and cortex was associated with ionomycin treatment and extensive changes in the soluble protein profile. These included the disappearance of vimentin, breakdown of spectrin and changes in alpha and beta crystallins. No changes were seen in the nucleus. A liquid fraction formed between the lens capsule and the main body of the lens with ionomycin treatment. The proteins in this liquid most closely resembled the cortex fraction. **Conclusions:** The lens fraction most affected by ionomycin treatment was the epithelium and the outer cortex. Spectrin and vimentin proteolysis and changes in crystallins were prominent features of this opacification model. The formation of a liquid fraction between the lens epithelium and outer cortex following ionomycin treatment indicated protein degradation in this region. These changes were consistent with calpain activation. However the limited changes in the inner cortex and the absence of changes in the nuclear fraction implied that ionomycin had not penetrated the interior of the lens.

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2896 - B449

Differential Gene Expression Induced in Human Lens Cells by Oxidative Stress

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Purpose: To understand the molecular basis of radiation-induced cataract, it is important to identify those genes with altered expression or function due to radiation-specific oxidative stress. Several published reports have investigated global genomic or proteomic changes in lens cells exposed to hydrogen peroxide, but it is unknown if this oxidative stresssor activates a response similar to ionizing radiation. We have used microarray analyses to investigate changes in gene expression in cultured human lens cells exposed to single acute doses of 55 MeV/amu protons. Protons are a predominant component of radiation encountered in space travel. **Methods:** A cultured human lens cell model (Blakely et al., IOVS 41:3808, 2000) was used for these studies. Lens cells were grown on matrix-coated plastic tissue culture dishes and irradiated with 55 MeV/nucleon proton beams at Berkeley Lab. Single doses of either 0.5, 2, or 4 Gy were given. RNA and protein were harvested at different times from 2 hr to 12 hr after radiation exposure. For gene array analysis, total RNA from an unirradiated control and an irradiated sample were reverse transcribed, PCR amplified and processed per directions provided by the manufacturer (Superarray Biosciences, Corp, MD). The biotin-labeled PCR amplicons were hybridized to functional cell cycle, p53 and ECM gene profiles, and applied to ECL film for fluorescent signal detection. Background noise signals were subtracted from the signal intensities for each gene spot and normalized to the positive housekeeping controls in each filter. Replicate experiments were completed. **Results:** Lens epithelial cells differentiating into fiber demonstrated an approximately two-fold increase in expression in a select number of genes. Irradiation of epithelial cells with 4 Gy protons demonstrated a nearly 3-fold increase in these genes within 2 hrs after exposure, whereas lens fiber cells showed significant decreased expression of these genes. **Conclusions:** Exposure to the oxidative stress of ionizing radiation from proton beams triggers premature expression of a select profile of cell cycle, p53, and ECM genes in cultured human lens epithelial cells, while decreasing their expression in differentiating fiber cells. Western analyses are in progress to confirm our findings at the protein level. The potential functional consequences of these radiation-induced changes will be discussed.

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Oxindolealanine as a Biomarker for Cataract Development in the Human Lens

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Purpose: Tryptophan oxidation is believed to play a key role in the lens matrix damage that ultimately results in the development of cataracts. Previous studies have suggested that oxindolealanine (OIA), an oxidation product of tryptophan, is present in human cataractous lenses. The purpose of this study was to confirm the presence of OIA in cataracts and to examine whether oxindolealanine is elevated in human cataractous lenses using LC/PDA/MS. **Methods:** Human lenses were obtained from several sources, including surgically removed whole human cataractous lenses and donor globes. Lens protein samples were hydrolysed in 6N HCl and analyzed by HPLC. System I consisted of a HPLC/PDA (Waters) and employed isocratic conditions on a Brownlee Spheri-5 C18 column with 20mM KH_2PO_4/10% methanol (pH 7) at 0.5 ml/min. Identification of OIA was confirmed by identical retention times and characteristic absorbance spectra from chemically synthesized OIA. A ratio was then expressed with respect to the phenylalanine peak for standardization. System II was used for definitive confirmation of OIA, and consisted of a LC/MS (ThermoElectron) with a Surveyor HPLC/PDA and Synergi Max-RP C12 analytical column. A gradient of 10% - 100% CH_3OH (0.1% TFA in water) with a flow rate of 0.2 mL/min was used. Mass spectra were recorded by a quadrupole ion trap mass analyzer (LCQ Advantage) equipped with an electrospray ionization source. Hydrolysates of calf lenses were used as a methods control. **Results:** OIA was detected by both chromatographic systems with Rt=19min (λ_{max}=250nm and 280nm shoulder) for system I and Rt=79.5min for system II (m/z =221). The mean content (+/- S.D.) of OIA (expressed as a ratio to phenylalanine) was 1.47 (+/- 0.48) in 10 human cataracts and 0.35 (+/- 0.08) in 4 aged (average 60 years) human controls, corresponding to a 4.2X increase ($p<.01$). OIA levels were also higher in the cataract nucleus (3.57 +/- 2.31) versus cortex (1.58 +/- 0.86) in five cataractous lenses, corresponding to a 2.26X difference ($p<.04$). Using the LC/MS system, OIA was confirmed in human cataract samples with an m/z = 221 and λ_{max}=250nm/280nm shoulder. OIA was not detected in calf lens hydrolysates, which served as a methods control. **Conclusions:** The results suggest that oxindolealanine is a useful biomarker for the determination of cataract development as well as being additional evidence for the role of tryptophan oxidation in the aging of the human lens.

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Dopamine Metabolites: Role in Age-Related and Post-Vitrectomy Cataracts

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Purpose: 1). To determine the levels of dopamine and biogenic amines in dense nucleus of Age Related Cataracts (ARC) and in aqueous humor (AH) and vitreous humor (VH) after vitrectomy with peeling of retinal membranes. 2). To determine the role of dopamine and derivatives on crystallines and lenses in culture. **Methods:** Nucleus from ARC and from autopsy eyes (New York Eye Bank) were digested with pronase in 0.1 M Tris Buffer ph 8.0, methanol/water extracted and phenol + fluorescent areas identified by TLC. In AH and VH of 4 patients post vitrectomy from retinal membranes; dopamine, other amines (DOPAC, HVA) and DOPAL (dehydroxyphenylacetaldelyde) was determine by GC-MS. Membranes of lens fibers and α, ß-M crystallines from humans and rabbit lenses were exposed to dopamine metabolites and tested for aggregation. Intact lenses were incubated in vitro in Dulbecco's media with dopamine or methyldopamine and tested for Rb^{86} transport and efflux, transparency and crystallin aggregations. **Results:** Dopamine, DOPAL, DOPAC and HVA levels in AH and VH increased significantly (255% to 760%) after retinal peeling indicating release of the amines from the retina. In ARC dopamine, HVA, DOPAC and DOPAL were identified in various fluorescent pigment of the dense cataract nucleus. Lenses incubated with 2mM methyldopamine for 5 days showed opacities, yellow-browning and changes in Rb^{86} transport (↓28 ± 3%) and efflux (↑82 ± 5%). Similarly, aggregation of crystallines took place when incubated with dopamine + MOA, or DOPAL but not with DOPAC or HVA. **Conclusions:** The fluorescent pigments of ARC contain dopamine derivatives including DOPAL which could result in crystalline aggregation and opacification. A role of these aldehydes and biogenic amines is postulated for nuclear sclerosis of ARC and nuclear lens opacities after vitrectomy for retinal peeling.

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Age-Related Compaction Affects Cortical Fiber Volume in Rabbit Lenses

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Purpose: Our prior investigations have shown that compaction is correlated with increased scatter as a function of age in rabbit lenses. Structural data also indicated that age-related compaction in outer nuclear fibers preceded compaction in the inner fibers. Therefore this study was conducted to determine if significant fiber compaction occurred in the cortex. **Methods:** New Zealand White rabbits at 16-20 months old (adult) and 3.5-4 years old (aged) were utilized for this study. Immediately after sacrifice, scatter was assessed by laser scan analysis using the Scantox™ In Vitro Assay System. Lenses were fixed, then dissected to obtain successive shells of cortical fibers. Straight fibers (lacking end-curvature) at 6 and 7 mm equatorial diameter were photographed and measured to determine fiber length, then processed and embedded for LM. Cross-sectional fiber area was measured in the equatorial segment using Scion Image (v. Beta 4.0.2). Estimates of fiber volume were made by multiplying fiber length by average cross-sectional area. **Results:** In adult lenses, the average cross-sectional area of fibers at 7 mm diameter was 33% smaller than at 6 mm diameter. Volume estimates showed a similar decrease of 30% from 6 to 7 mm. In aged lenses, there was a 48% decrease in the average cross-sectional area of fibers at 7 mm diameter as compared to those at 6 mm diameter. The volume of aged fibers decreased approximately 44% from 6 to 7 mm diameter. Thus, as expected, the older, more centrally located fibers were larger than younger, more peripherally located fibers in both adult and aged lenses. Comparisons between age groups revealed that at a diameter of 6 mm, the volume of aged fibers was 15% smaller than adult fibers. Furthermore, aged fibers at 7 mm diameter displayed a decrease in volume of 31% as compared to adult fibers at the same diameter. **Conclusions:** Cortical fibers in aged lenses had markedly reduced volume as compared to those in adult lenses, indicating a greater degree of compaction. In addition, it appears that younger, more peripheral fibers undergo more compaction than older, more central fibers in rabbit lenses. The data suggest that increased scatter in aged rabbit lenses is primarily due to cortical fiber compaction which may result from water loss and subsequent volume reduction.

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Amyloid-Like Fibrillar Pattern in the Adult Lens Nuclear Cytoplasm of OXYS Rats Appears to be a Reorganization of Crystallins

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Purpose: To characterize the previously described (Marsili et al., Exp. Eye Res. 79, 595-612, 2004) unusual pattern of fibrils in a narrow band of adult nuclear fiber cells of the OXYS strain of rats. **Methods:** Lenses from OXYS rats, which have an inherently high level of oxidative stress, and normal control Wistar rats were Vibratome sectioned fresh, immersion fixed and prepared for light and electron microscopy. Histological sections were stained for up to 20 hrs with Congo Red. **Results:** Electron microscopy revealed a region 400-500 µm from the capsule of OXYS lenses containing fiber cells with cytoplasm composed of a complex tangle of fibrils that were similar in many ways to amyloid fibrils. Two types of patterns were observed. For some fiber cells the entire cytoplasm appeared to be replaced by the fibrillar network surrounded by typical plasma membrane. In other fiber cells a localized region of cytoplasm displayed the fibril pattern that blended into normal appearing cytoplasm predominated by closely packed 16-20 nm alpha-crystallin spheres. Again typical plasma membranes surrounded the cells and did not appear structurally to be involved in the formation of the fibrils. Some of the localized domains appeared to have a nucleation center from which fibrils emanate. Linear fibrils found at the interface with normal cytoplasm were particularly interesting. They were often up to 200 nm in length and composed of a linear string of 16-20 nm diameter beads. A stain excluding region connecting the beads suggests a mode of formation from alpha-crystallin spheres. Fibril interiors excluded heavy metal stain; however, fibril surfaces and interfibril spaces stain like typical hydrophilic proteins. Fibrils were often straight for short distances but were more commonly curved. Fibril diameters varied over a narrow range with most around 7-8 nm and fibrils were frequently branched. True amyloid fibrils are usually straight and not branched. Moreover, histological sections showed no indication of Congo Red staining or birefringence. **Conclusions:** These morphological observations suggest that the fibrils are formed from crystallins modified by oxidative damage. Similar in appearance to amyloid fibrils, the fibrils probably result from the alignment of hydrophobic domains of modified alpha-crystallin spheres producing stain excluding interiors.

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Structure/Function Characterization of Post-Trabeculectomy Induced Lens Changes in Aged Rabbits

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Purpose: Trabeculectomy, generally considered the standard "filtration" type of surgery for open-angle glaucoma, very frequently leads to cataract. We have previously reported that 1.5 months post-trabeculectomy performed on young rabbits (3 months old) an abnormal suture branch oriented directly toward the surgical site is formed that causes a quantifiable negative effect on lens optical quality. The purpose of this study was to determine if a comparable trabeculectomy induced structure/function compromise would occur in older rabbits, a population more representative of the human condition. **Methods:** Trabeculectomy was performed on retired breeder Dutch-belted rabbits (n=10) that were at least 5 years of age. Age-matched un-operated rabbits were used as controls (n=10). One and half to three months after surgery, lens function (spherical aberration [avg. back focal length, BFL] and sharpness of focus [variability in BFL, BFLV]) was quantified by low power helium neon laser scan analysis. These results were then correlated with lens structure as assessed by light, scanning electron microscopic and CAD reconstructions of lenses derived from micrographs. **Results:** Structural analysis demonstrated that the lens from "each" operated eye had an abnormal sub-branch that extended from the normal, age compromised crooked posterior line suture directly toward the surgical site. While the surgically induced formation of an abnormal suture branch 6 weeks after surgery did not effect a significant compromise in lens optical quality (control lenses: BFL = 16.89 ± 0.31mm and BFLV = 0.43 ± 0.03 vs. 6 week post surgery lenses BFL = 16.32 ± 0.15 and BFLV = 0.51 ± 0.06), 12 weeks after surgery a significant compromise had occurred (12 week post surger lenses BFL = 17.29 ± 0.38 and BFLV = 0.62 ± 0.06). **Conclusions:** Abnormal suture formation following disruption of the environment via trabeculectomy results in optical compromise in both young and aged rabbits but at a different time course. These sutural changes appear to be characteristic of early changes in lens structure that ultimately result in cataract formation. Prevention of these early sutural changes by inclusion of factors that promote normal fiber development and growth following surgery, even in older subjects may lead to a better surgical outcome.

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2902 - B455

Upregulation of Lens VEGF in a Rat Model With Persistent Hyaloid Vasculature

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Purpose: A spontaneous mutation (Nuc1) in the Sprague-Dawley rat causes a number of eye-specific pathologies, including persistent fetal vasculature (PFV). Since the level of vascular endothelial growth factor (VEGF) in the lens may be involved in the normal regression of the fetal vasculature, we have investigated VEGF expression in the Nuc1 lens.
Methods: Expression of VEGF in the lens of Nuc1 and age-matched wildtype rats was assessed by western blotting with VEGF specific antibodies. Localization of VEGF expression was determined immunohistochemically on frozen sections of eyes from Nuc1 and wildtype rats.
Results: Immunohistochemical analysis of Nuc1 and wildtype eyes from rats 5 weeks of age using VEGF specific antibodies revealed little or no detectable signal in wildtype lens whereas strong immunofluorescence was present in the lens tissue (both fibers and epithelial cells) of Nuc1. At this stage the fetal vasculature has completely regressed in the normal eye, but in Nuc1 it remains intact. Western blotting confirmed that VEGF is present at much higher levels in the mutant lens than in the wildtype lens and also indicated that in Nuc1 multiple isoforms of VEGF are expressed.
Conclusions: The data are consistent with the idea that persistence of the fetal vasculature of the eye may be associated with elevated levels of VEGF in the lens. Additional work is needed to establish that a cause and effect relationship exists between these two events. The Nuc1 model provides opportunities to investigate this and other aspects of the pathophysiology of PFV.
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2903 - B456

Fluorescence Profiles of Hyperbaric Oxygen/UVA Treated Guinea Pig Lenses, GPX-1 Knock Out Rat Lenses and Clear Human Lenses

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Purpose: Fluorophores may contribute to or be markers of cataract. Lenses from guinea pigs (GP) treated with hyperbaric oxygen and treated with chronic UVA light, as well as lenses from glutothione peroxidase-1 knockout rats (GPX1-ko) have shown signs of oxidative damage. By using fluorescent spectroscopy, lens flourophores from human donors were compared to lens flourophores from rat and GP models.
Methods: GP lenses from control and treated (66 treatments of hyperbaric oxygen and chronic UVA light over 22 weeks) groups, were obtained at age about 23.5 months. Each GP lens was dissected into nuclear, equatorial and cortical regions. Fifteen month old rat lenses were obtained from GPX1-ko and controls. Human lenses (31-74 y) were separated into cortical and nuclear regions. After homogenization in methanol, fluorescence excitation/emission/intensity spectra were measured with an emission range of 200-650 nm and an excitation range of 200-800 nm.
Results: Three peaks were prominent in the 3D excitation/emission fluorescence spectra of rat, GP, and human lens protein suspensions. Peak I near 295/340 (nm excitation/emission) was the most consistent and prominent peak. Peak II 360/445, a diagonal ridge Peak III 265/575, and Peak IV 470/520 were present in all species. Fluorescence spectra from rats and GP were more similar to each other than to human lens spectra. For human data, in relations to Peak I, Peak II was about 75 %. Peak III was about 17 times larger, and peak IV was 10% of Peak I. For the rat and GP data, in relations to Peak I, Peak II was about 0.5%, and Peak IV was about 2%. Peak III varied among the GP and rat lenses. The intensity of Peak II relative to Peak I was smaller in paired controls, consisting of untreated GP and native rats, relative to treated GP and GPX1-ko rats. This was true for all 3 regions of the GP lens. The ratio Peak II/Peak I increased with age in the human lenses. The relative intensities of Peaks III and IV were higher in all 3 regions of treated GP lenses and were higher in GPX1-ko samples compared to controls. Peaks III and IV decreased with increasing age in human lenses.
Conclusions: The advantage of 3D excitation/emission spectra over conventional 2D fluorescence spectra is that all changes in the fluorescence characteristics can be quantified that could be potentially missed in 2D spectra. By comparing 3D fluorescence spectra from human and model systems, we may begin to determine the fluorescent components that are markers of oxidation and cataract.
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2904 - B457

Age-Related Cataract in Anti-Oxidant Altered Mice

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Purpose: To examine the effect on age-related cataract of removing or diminishing, versus increasing, specific anti-oxidant enzymes or homonal stimulation in the lens.
Methods: In separate groups mice were deprived homozygously of GPX1, or heterozygously of SOD1, or both, by homologous recombination knockouts, or had additional catalase added only to the mitochondria by targeted transcarbamylase leader sequence. All mice were then bred back at least 10 generations onto the C57BL/6 strain background. In addition, mice on an outbred mixed background with knockout of the growth hormone receptor (GHRKO) and no circulating IGF1 were separately prepared. The mice were then examined at 4-5 months or 21-30 months by hand held slit lamp use by a single blinded observer. Cataracts were graded progressively as 0 to4+, with the latter representing a mature cataract.
Results: Mutant mice with no GPX1 developed graded cataracts that were significantly advanced over that of controls in late life; Mice with 50% of normal levels of SOD2 did not differ from controls; mice bearing both deletions developed cataracts with the same age-related advancement as the GPX1 KO mice alone, with no added effect from the half-reduced SOD2. Mice with catalase added only to the mitochondria showed a significant delay in cataract development at 15-17 months of age, but this advantage was reduced to p=.06 by 24 months and lost entirely by 30 months. Mice with knockout of the GH receptor (GHRKO) had significantly less advanced cataracts in old age.
Conclusions: These findings provide support for the oxidative causality of age-related cataract development, with the notable exception of the SOD2 heterozygous KO mutant. It is noted that 50% of the normal level of SOD2 may be sufficient to provide the adequate activity of SOD2, i.e., the conversion of O* to H202 in the lens. The cataract delay in the GHRKO mice are an example of the protection from oxidative damage provided by the removal of circulating IGF1, as has been seen in other studies testing tissues and the whole mouse against oxidative challenges. These studies empasize the interplay of specfic genes and the environment in the development of age-related cataract.
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2905 - B458

Characterization and Non-Invasive Imaging of Lens ß-Amyloid in the Tg2576 Mouse Model of Alzheimer's Disease

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Purpose: Cerebral ß-amyloid (Aß) accumulation is a cardinal feature of Alzheimer's disease (AD). We have previously identified Aß deposition, amyloid pathology, and co-localizing supranuclear cataracts (SNC) in human AD lenses (Goldstein et al., Lancet, 2003). Similar lens pathology and Aß overexpression have been reported in Tg2576 mice (Goldstein et al. [2001] 42: ARVO Abst 1614; Render et al. [2003] 44: ARVO Abst 3063), an established AD mouse model (Hsiao, *Science* [1996] 274:99). In AD lens, Aß co-aggregates with alphaB-crystallin (and other cyoplasmic proteins) as e- dense cytosolic aggregates in supranuclear fiber cells. Similar molecular pathology may be detectable *in vivo* in Tg2576 mice. **Methods:** Immunohistochemistry/fluorescence, quantitative western blot, SELDI-MS, tryptic digest/MS sequencing, immunogold EM, EM autometallography; stereophotomicroscopy. Non-invasive *in vivo* techniques: static and quasi-elastic light scattering (SLS,QLS), fluorescent ligand scanning (FLS). **Results:** Human Aß/ß-APP are overexpressed in Tg2576 mouse lens. Cytosolic e- dense Aß aggregates are present in Tg2576 but not WT fiber cells. Tg2576 mice showed morphologically variable SNC phenotypic expression. Non-invasive quantitative *in vivo* lens analyses were accomplished using static light scattering (SLS, Seeberger *et al.*, J Biomed Opt. [2004] 9:116) and a more sensitive quantitative technique, quasi-elastic light scattering (QLS, Optiscan 1300, Neuroptix, Amherst, MA). Systemic administration of a fluorescent lipophilic amyloid-binding ligand (Me-X04, Klunk *et al. J Neuropathol Exp Neurol* [2002] 61:797) in Tg2576 and amyloid-bearing 87V Prp-infected mice resulted in supranuclear Me-X04 binding *in vivo.* Supranuclear Me-X04 histofluorescence, Aß immunostaining, and amyloid pathology were also observed in *ex vivo* lens specimens. Preliminary results suggest that Aß pathology may be present and detectable earlier in lens than in brain. **Conclusions:** Tg2576 mice express human Aß in the lens and exhibit SNC pathology. Tg2576 mice afford an ideal animal model for non-invasive detection and quantitative *in vivo* monitoring of Aß-associated lens pathology.
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2906 - B459

Alzheimer's Disease β-Amyloid (Aβ) Mediates Metal-Dependent Human Lens Protein Aggregation and Light Scattering

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Purpose: Cerebral β-amyloid (Aβ) accumulation is a cardinal feature of Alzheimer's disease (AD). We have previously identified Aβ, amyloid pathology, and co-localizing equatorial supranuclear cataracts (SNC) in human AD lenses (Goldstein et al., *Lancet*, 2003) and noted similar SNC phenotype and lenticular human Aβ overexpression in Tg2576 transgenic mice (Goldstein et al., IOVS 2001; 42: ARVO Abst 1614). Lens Aβ accumulates within equatorial fiber cells as e- dense cytosolic aggregates. Aβ binds cytoplasmic lens proteins including αB-crystallin. Aβ and αB-crystallin co-localize in the cytosol and co-aggregate as birefringent congophilic deposits. Since Aβ sequesters copper and zinc in cerebral neuritic plaque and both metals promote Aβ aggregation, we hypothesized that metalloprotein interactions may be involved in Aβ-mediated lens protein aggregation. To test this hypothesis, we (1) performed metal mapping analyses in unfixed, cryopreserved human and Tg2576 lenses, and (2) studied the metalloprotein biochemistry of Aβ-mediated lens protein aggregation.
Methods: Cryofixation/section, synchrotron X-ray fluorescence microscopy, EM autometallograpy, ICP-mass spectrometry, metal histochemistry, atomic force microscopy, quasi-elastic light scattering, co-immunoprecipitation, crosslinkage analysis.
Results: Copper, iron, and zinc are present in the cytosolic fraction of aged human lenses (1.8, 2.0, 27 μg/g protein, respectively). Aβ and metal (especially zinc) co-localize within the cytoplasm of equatorial supranuclear lens fiber cells. Human Aβ42 potently and specifically promotes metal-dependent lens protein aggregation and increased light scattering. Aβ-mediated lens protein aggregation is critically dependent on metalloprotein redox chemistry.
Conclusions: Lens Aβ promotes hetero-oligomeric cytosolic lens protein aggregation *via* protein-protein interactions and metalloprotein redox reactions. Once formed, Aβ-associated lens protein aggregates scatter light that manifest as the SNC phenotype. We hypothesize that SNC and its molecular antecedents are early, organ-specific expressions of the same underlying AD disease process evident in the brain.
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2907 - B460

***In vivo* and *in vitro* Cataract Thresholds in the Lens during 7.8 MHz Ultrasound Exposure**

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Purpose: Regulatory bodies such as the U.S. Food and Drug Admnitration have long-standing standards for diagnostic ultrasound exposure levels. Color flow and continuous wave Doppler instrumentation can readily exceed these thresholds in ocular examinations. Our aim was to study the formation of cataracts in lenses exposed to ultrasound at a frequency of 7.8 MHz, similar to that in some Doppler instruments.
Methods: We used a spherically focused ultrasound transducer with an F-ratio of 1.1 and operated at its 5th harmonic, 7.8 MHz. After calibration to determine the relationship between excitation voltage and radiation force, we exposed rabbit eyes in vivo to various power levels. Lenses were observed during exposure and histology obtained subsequently. We also exposed cow lenses in vitro in a temperature regulated waterbath, with implanted thermocouples used to measure temperature levels during exposure and a fibre optic endoscope used to record cataract formation.
Results: In vivo, cataracts always formed at a power level of 1900 W/cm^2 (mean time = 28 sec), with cataracts forming in about half of cases at 1200 W/cm^2(mean time >3 minutes). In vitro, we found a rapid temperature rise (time constant = 7 seconds) in the focal zone. Cataracts appear to have formed at temperatures of 45^{o}C or higher. In vitro cataracts tended to require somewhat higher intensities than in vivo (minimum 1900 W/cm^2), but shorter exposure durations.
Conclusions: Current standards for ultrasound exposure are more stringent for ophthalmology than any other clinical specialty. The primary concern is thermally mediated cataract formation due to the high attenuation coefficient of the lens and its lack of a blood supply. Our results suggest a wide safety margin of on the order of a factor of 50 with current standards. As reduced exposure levels involve some reduction in diagnostic capability, our findings suggest that less stringent standards would be of benefit for ophthalmic ultrasound, including Doppler.
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2908 - B461

Estimates of Local Refractive Index Variations Across Micrometer Sized Particles in Human Age-Related Nuclear Cataracts

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Purpose: To employ quantitative electron microscopy to determine the refractive index, which is related to protein concentration, in and around multilamellar bodies thought to be potential sources of excess forward scattering in human age-related nuclear cataracts.
Methods: Based on the procedures of Lamvik and Davilla (J.Elec.Micr.Tech. 11, 97-101, 1989), who used polystyrene spheres as electron optical wedges of known mass density, we have examined evaporated carbon films of varying thickness to derive a scale of transmittance (integrated intensity) as a function of electron path length. The scale relates optical density of transmission electron micrographs to carbon concentration using the measured film thickness and the known mass density of amorphous carbon (1.9-2.0 g/cc). The optical density of micrographs of well-preserved, unstained fiber cell cytoplasm from normal and cataractous human lens nuclei were compared to the derived scale to estimate protein concentration and local refractive index. Low dose techniques were used to minimize beam damage.
Results: The measurement of mass in the transmission electron microscope depends on the accurate determination of the decrease of electron flux through homogenous protein gels. Digital bright field images were used with integration of the intensity to give a reproducible measure of the electrons lost and the molecular concentration. A difficulty with thin sections is the correction for the embedding medium, such as epoxy resin, which has taken the place of water in the fixed tissue. Therefore only approximations to native refractive index are possible and the best comparisons are made within the same thin sections in adjacent regions. Cytoplasm from nuclei of human normal and cataractous lenses give similar values and are consistent with refractive index measurements of 1.40 Å± 0.01. Refractive index measurements of cytoplasm within 1-4 Åµm diameter multilamellar bodies have been found to be similar to the surrounding cytoplasm for some particles and different by 2-5% for others.
Conclusions: Measurements of mass and estimated refractive index in fixed, embedded, unstained thin sections of human nuclear fiber cells suggest that the refractive index is constant among fiber cells in each developmental region. Some multilamellar bodies have been observed to have different interior refractive index, supporting the suggestion that they should be considered as potential sources of excess nuclear light scattering in cataracts.
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2909 - B462

***In vivo* Fluorescence Imaging of Mouse Lenses**

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Purpose: To develop a simple, *in vivo* imaging for slit lamp observation in mice. Introduction: Enhanced Green Fluorescent Protein (EGFP, from Clonetech Laboratories, Inc.) is a common label for protein expression in vivo. Mice expressing an EGFP- labeled expression product were examined using emission and barrier filters with selected wavelengths to observe spatial distributions in living animal lenses.
Methods: Unanesthetized mice were examined using a slit lamp. Eyes were dilated with a drop of 0.5% tropicamide, 5% phenylephrine solution and held in front of a slit lamp (Nikon FS-2). Slit lamp examinations, at 40X magnification, were recorded using an integrated digital video camera. Three absorption filters were utilized in the examination. An excitation filter allows a narrow range of wavelengths (465-498nm) to excite EGFP (max: 488) in the lens. An emission filter (515-560nm), was used with the excitation filter, for transmission of EGFP emission (max: 510nm). For visualization of opacity without interference of EGFP emission, a barrier filter prevented EGFP transmission (475-560nm). Frames were selected from video recordings, using Adobe® Premier®, saved in .tif file format, and then cropped and oriented using Adobe® Photoshop®.
Results: Anterior views were used to observed product expression in the lens. Slit lamp views allowed detailed observation of the lens. Opacities in the subnuclear cortex and the nucleus of the lens were apparent in the slit view without filters. Using the excitation filter, expression of the transgene was observed mostly in the subcortical region of the lens. With excitation and emission filters, EGFP expression was observed in the subcortical region of the transgenic mice. The slit view with the EGFP blocking filter confirmed that there was opacity that corresponded with position and pattern of product expression.
Conclusions: Slit lamp fluorescence imaging is a simple and rapid method for the evaluation of EGFP expression products associated with opacity in the living mouse lens. EGFP excitation and emission filters were necessary for distinguishing the expression of EGFP-labeled product from normal lens constituents seen without filters. The EGFP blocking filter was necessary to distinguish total expression from aggregated EGFP labeled product.
CR: E.E. Arnett, None; **T.M. Seeberger**, None; **A.V. Crum**, None; **J.I. Clark**, None.
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2910 - B463

Image Management System for Correlation of Temporal and Spatial Variation in Mouse Lens Phenotype

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Purpose: Systematic examinations of the progressive changes in lens transparency during lens opacification in transgenic mice results in an enormous data base of digital slit lamp images. Analysis of individual images and recognition of patterns in lens opacification with age is expensive in time and effort. The objective of the current project is to develop software that manages the large data base and facilitates comparative analysis of variations in the phenotypes observed in transgenic mice.
Methods: Unanesthetized mice were gently held in position for examination with the slit lamp. Pupils were dilated with one drop of 0.5% tropicamide, 5% phenylephrine hydrochloride ophthalmic solution. Slit lamp (Nikon FS-2) examinations were approximately 1 to 2 minutes and were recorded using a digital video camcorder (Canon Optura 20). The records were observed using Adobe® Premier® 6.0 software and selected images from each examination were stored in .tif file format. Stored images were cropped and oriented using Adobe® Photoshop® and entered into the image management database.
Results: The web-based software is implemented on top of WIRM (http://www.wirm.org), an open source experimental management system initially developed for managing brain mapping data. The mouse eye image files are stored on the server's file system and the attributes that describe the context of the images are stored in a relational database. The software system helps manage the extensive data and allows efficient comparisions of selected views that facilitate image analysis and pattern recognition. Information about the images, including genetic information about the subject, can be easily retrieved for any images of interest.
Conclusions: The image management system decreased the time required to input, organize and evaluate image files recorded using the slit lamp. This system will benefit the rapid analysis of phenotypes in databases storing thousands of images. Collaboration between laboratories in across the institution or throughout the world is simplified. Interfaces to a variety of common software programs including Adobe® Photosystem® and Power Point® make it convenient for presentation, pattern recognition and analysis.
CR: C.T. Fong, None; **E.E. Arnett**, None; **J.F. Brinkley**, None; **J.I. Clark**, None.
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2911 - B464

Optiscan 2400, a New Clinical Instrument for Non-Invasive Measurement of Quasi-Elastic Light Scattering in Human Lens *in vivo*

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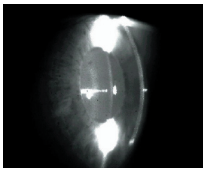
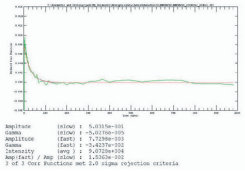
Purpose: To characterize extent & rate of lens protein aggregation using a new, non-invasive ophthalmic instrument for quantitative quasi-elastic light scattering (QLS) measurements in discrete regions of human lens *in vivo*.
Methods: The Optiscan 2400 was designed to capture QLS in the lens. The instrument uses a low-wattage Class 1 laser light (780 nm), photon detector, autocorrelator, and eye-motion compensation technology.
Results: Images of Optiscan 2400 in operation and representative autocorrelation functions from lens QLS analyses on human subjects are presented.



Conclusions: Previously, we reported the identification of Alzheimer's disease (AD), beta-amyloid (AB), AB-containing cytosolic aggregates, birefringent congophilic amyloid pathology, and unique co-localizing supranuclear cataract in *ex vivo* lenses of patients with advanced AD (Goldstein et al., Lancet, 2003). Similar cataracts and lenticular AB overexpression have been reported in the Tg2576 mouse model of AD (Goldstein *et al*., IOVS 2001;42:ARVO Abst 1614; Render *et al*. IOVS 2003;44:Abst 3063). The Optiscan 2400 affords unique and sensitive means for non-invasive quantiative detection and monitoring of protein aggregation in discrete regions of human lens *in vivo*. Potential applications of this technology also include assessment of age-related and drug-related cataract formation as well as screening for cataractogenic potential of systemically administered drugs.



CR: L.T. Chylack, Neuroptix Corporation I, C, P; **L.E. Goldstein**, Neuroptix Corporation I, C, P; **N. Ford**, Neuroptix Corporation I, E, C; **J.I. Clark**, Neuroptix Corporation I, C; **A. Green**, Neuroptix Corporation I, C; **P. Hartung**, Neuroptix Corporation I, E, C; **R. Pineda**, Neuroptix Corporation F.
Support: Neuroptix Corporation

2912 - B465

Models of Human Cataract Validate Shack-Hartmann Nuclear Cataract Assessment

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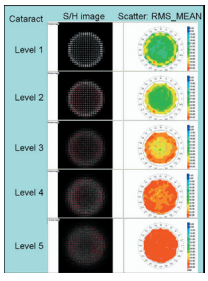

Purpose: To use physical and computer models to suggest ways to improve metrics of nuclear cataract severity derived from Shack-Hartmann (S/H) images by optimizing exposure times and pupil size.
Methods: A physical model eye consisting of a fixed lens, variable pupil, cuvette, and retina was mounted to an S/H wavefront sensor. Located behind the pupil (retina side), the 5mm cuvette containing one of five polystyrene microsphere solutions served to vary nuclear cataract severity. The computer model was built in ZEMAX based on an exact replication of the physical eye model/wavefront sensor combination. We used these models to test the influence of exposure and pupil size on previously reported scatter metrics [JRS, 2004, 20(5), S515]. Exposure times were varied between 100ms and 200ms and pupil diameters were varied between 5mm and 7mm.
Results: In both models, as cataract density increased light transmission decreased and scattered light increased. When pupil and exposure were allowed to vary, the forward scatter metric RMS_MEAN explained 59% of variance in simulated cataract severity. The coefficient of determination was improved to 64% by holding pupil size constant. When pupil size varied and exposure was held constant RMS_MEAN explained 61% of variance. By holding pupil diameter and exposure constant RMS_MEAN explained 71% of the variance in cataract severity.
Conclusions: The ability of S/H scatter metric RMS_MEAN to monitor changes in model cataract severity is significantly increased by holding pupil diameter and exposure time constant. The optimal exposure time should be selected to exploit the dynamic range of the camera without saturating the image and be fast enough to minimize eye motion blur.
CR: W.J. Donnelly, None; **R.A. Applegate**, University of Texas Health Science Center at San Antonio P.
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2913 - B466

Lens Stress Forces

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Purpose: To investigate the spatial variation of biomechanical forces in the human lens.
Methods: The superficial anterior human lens was modeled using thin membrane theory. An ellipsoid of revolution was used to represent the human lens, of 8 mm diameter and 1.6 mm sagittal half-thickness. Radial (meridional, N_ϕ) and hoop forces (N_θ) were calculated and normalized to internal lens pressure.
Results: Radial forces were tensile from the anterior lens pole to the equator (N_ϕ>0), dropping nonlinearly from a maximum at the pole to one-third maximum at the equator. Hoop forces were initially tensile (N_θ>0) in the central anterior lens, dropping to zero at 22.7° azimuth angle (central 6.8 mm dia), and became compressive towards the equator (N_θ<0). The absolute ratio of pole:equator hoop forces exceeded 2.
Conclusions: These results indicate that cells in the central 6.8 mm dia anterior zone of the lens are subjected to tensile radial and tensile hoop forces. This dual tensile loading would favour centrifugal migration of lens epithelial cells. During accommodation these tensile forces could exert significant moulding forces on the epithelium and superficial cortex. Nearer to the lens equator, lens cells experience tensile radial forces, but with compressive hoop forces. These combined forces would favour radial alignment and may contribute to the curious ordering of lens fibres in radial columns. The radial and hoop forces in a thin shell are influenced by lens thickness and equatorial diameter. Thus, the spatial variation of N_ϕ and N_θ may be affected by aging.
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