528 Molecular pharmacology and ocular toxicology
Thursday, May 11, 2017 11:30 AM–1:15 PM
Room 301 Paper Session
Program #/Board # Range: 5587–5592
Organizing Section: Physiology/Pharmacology

Program Number: 5587
Presentation Time: 11:30 AM–11:45 AM
Vitamin D in Systemic Sclerosis Patients with Dry Eye Syndrome
Caterina Gagliano[1, 2], Rosario Foti[3, 2], Elisa Visalli[3, 2], Roberta Amato[1, 2], Giuseppe Calì[4, 2], Giovanni Panta[5, 2], Daniela Rocca[5, 2], Alessandra Pizzo[4, 2], Giorgio Amato[3, 2], Giulia Malaguarnera[1, 2]. [1]Ophthalmology, NEST (Neurovisual Science Technology), Catania, Italy; [2]Rare Disease Center (Ra. Di.Ce.), Santa Marta Hospital, Catania, Italy; [3]Rheumatologic Unit, Catania University, Catania, Italy; [4]Eye Clinic, Catania University, Catania, Italy; [5]Eye Clinic, Santa Marta Hospital, Catania, Italy.
Purpose: The purpose of this study was to explore the clinical and pathogenic significance of vitamin D (25-hydroxyvitamin D) in systemic sclerosis (SSc) patients with dry eye syndrome (DES). The relationship between the severity of DES, SSc disease activity and levels of 25-hydroxyvitamin D was investigated.
Methods: In this cross-sectional study, 32 consecutive patients with SSc and DES were enrolled. We measured blood concentrations of 25-hydroxyvitamin D (25 OH D) and correlated the results with SSc disease activity calculated with modified Rodman Skin Score (mRSS), Tear osmolarity measurements (TearLab system), Tear Break-Up Time (TBUT) test, and Schirmer tests (type I and II).
Results: Levels of 25 OH D were decreased in all SSc patients with DES as compared to normal controls and the reduced levels of 25 OH D were stable over the observed period of 2 months. Levels of 25 OH D correlated inversely with mRSS score and tear osmolarity ($r = 0.610$, $p < 0.001$), positively correlated with Schirmer values, ($r = -0.231$, $p = 0.045$), and TBUT values ($r = -0.325$, $p = 0.007$) among all patients.
Conclusions: Our study demonstrated a high relationship with SSc disease acitivity, severity of DES and low levels of 25-hydroxyvitamin D.
Commercial Relationships: Caterina Gagliano; **Rosario Foti**, None; **Elisa Visalli**, None; **Roberta Amato**, None; **Giuseppe Calì**, None; **Giovanni Panta**, None; **Daniela Rocca**, None; **Alessandra Pizzo**, None; **Giorgio Amato**, None; **Giulia Malaguarnera**, None

Program Number: 5588
Presentation Time: 11:45 AM–12:00 PM
Perfluoro-n-octane cytoxicity in porcine neuroretina organotypic culture
Ivan Fernandez-Bueno[1, 2], Girish K. Srivastava[1, 3], M L. Alonso-Alonso[1], Maria Teresa Garcia-Gutierrez[1], Manuel Gayoso[4], Rosa M. Coco[1], Jose-Carlos Pastor[1, 2]. [1]IOBA - University of Valladolid, Valladolid, Spain; [2]Red Tematica de Investigacion Cooperativa Sanitaria (RETICS), Oftared, Instituto de Salud Carlos III, Valladolid, Spain; [3]Centro en Red de Medicina Regenerativa y Terapia Celular, Junta de Castilla y Leon, Valladolid, Spain; [4]Cell Biology, Histology and Pharmacology, University of Valladolid, Valladolid, Spain.
Purpose: The supposed toxicity in patients of a perfluoro-n-octane (PFO; Alaocta®, Ala Medics®, Germany) originated a sanitary alert in Spain. This PFO had been considered non-toxic by routine in vitro cytotoxicity assays following UNE EN ISO 10993-5 and -12: 2009. Our proposal evaluates the acute cytoxicity of this sanitary product in an organotypic culture of porcine neuroretina.
Methods: Neuroretina explants were obtained from fresh porcine eyes and exposed to suspected PFO (n=6) for 30 min. PFO were named according to the packing date (DD/MM/YY). Negative and positive controls were exposed to PFO from other manufacturers and to phenol (1.6 mg/ml), respectively. After exposure, explants were cultured for 72 hours. Unexposed controls were cultured in parallel. Each experiment was performed in triplicate. Samples were processed for epoxy resin embedding. Semithin sections (1μm) were stained with toluidine blue and evaluated by light microscopy. Subjective scoring of tissue/cell modifications was performed (grade 0 to 4). Numerical ranking greater than 2 is considered cytotoxic according to UNE EN ISO10993-5: 2009.
Results: Fresh samples showed preserved neuroretina architecture with initial modifications (grade 0-1). Unexposed and negative controls presented partial loss of photoreceptor and incipient retina vacuolization (grade 1) at 72 hours of culture. Positive controls revealed total retina degeneration (grade 4). Neuroretina explants exposed to Ala Medics® PFO and cultured 72 hours showed different degenerative patterns: low (grade 2; PFO200114), mild (grade 3; PFO150414, PFO050514 and PFO070714), and severe (grade 4; PFO061014 and PFO171214).
Conclusions: Ala Medics® PFO time-dependent cytoxicity (grade>2) has been confirmed in porcine neuroretina cultures. This study shows that ISO routine premarket methods used to detect PFO toxicity have failed, and proposes a suitable and more sensitive method to determine intravitreal medical devices potential toxicity.
Commercial Relationships: Ivan Fernandez-Bueno, None; **Girish K. Srivastava**, None; **M L. Alonso-Alonso**, None; **Maria Teresa Garcia-Gutierrez**, None; **Manuel Gayoso**, None; **Rosa M. Coco**, None; **Jose-Carlos Pastor**, None
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Program Number: 5589
Presentation Time: 12:00 PM–12:15 PM
Ocular Complications Following Intracameral Injection of an Elevated Dose of Cefuroxime During Phacoemulsification Surgery
Andrew Melchioris, Michael Zgrabik, Joan Hornik, David G. Miller. Retina Associates of Cleveland, Cleveland, OH.
Purpose: Intracameral injection of cefuroxime sodium (1 mg/0.1 mL) has been reported to reduce the risk of endophthalmitis following cataract surgery and is becoming more common. This is a compounded medication, which can lead to rare dilution errors. This study describes the results of 10 times elevated doses of intracameral injection of cefuroxime sodium. Dilution errors of this type may be more common with the adoption of cefuroxime endophthalmitis prophylaxis.
Methods: We retrospectively analyzed 7 eyes of 7 patients who were operated on the same day by the same surgeon. At the end of surgery, an incorrect dose of 10 mg in 0.1 mL cefuroxime (30 mg/mL, GlaxoSmithKline) was injected into the anterior chambers of 7 eyes. Incorrect dilution of cefuroxime was noticed at the end of operation day. The patients were seen at 1 day, 1 week, and 1 month follow-ups by the same physician, and the best corrected visual acuity, macula, and intraocular inflammation of each patient were closely observed. Visual acuities were converted to logMar. Comorbidities were also tabulated.

Results: The preoperative mean visual acuity was logMar 0.77 (20/120). On postoperative day 1, the mean visual acuity in logMar was 1.40 (20/500 snellen) showing a loss of 0.63 units. On the postoperative day 1, 6/7 patients (86%) were diagnosed with cystoid macular edema, and 3/7 patients (43%) were found to have excessive (2+) cell/ flare. At one week, the mean logMar was 0.54 (20/70), and macular edema and excessive inflammation resolved in all cases. Final visual acuity mean at 1 month was 0.44 logMar (20/55), a 0.96 logMar improvement from postoperative day 1 visual acuity, and a 0.33 improvement from preoperative visual acuity. Preexisting ocular comorbidities (5 with age related macular degeneration, 1 with diabetes mellitus, 2 with epiretinal membrane, and 1 with primary open angle glaucoma) were present in 5/7 patients (71%), and remained stable.
Conclusions: Compounded intracameral injection of cefuroxime sodium at a dose of 10 mg/0.1 mL (10 times normal) was associated with diminished acuity, cystoid macular edema, and excessive intraocular inflammation resolving largely within 1 week in this study. Elevated doses of cefuroxime, occurring from dilution errors in compounding this medication, appear to lead to ocular morbidities, which are self limiting.
Commercial Relationships: Andrew Melchioris; **Michael Zgrabik**, None; **Joan Hornik**, None; **David G. Miller**, None

Program Number: 5590
Presentation Time: 12:15 PM–12:30 PM
Identification of the hnRNPL and LOXL1-AS1 lncRNA complex: Implications for pathology in exfoliation glaucoma
William M. Johnson[1], Inas F. Aboobakar[1], Laura Finnegan[2], R Rand Allingham[1, 3], Michael A. Hauser[4, 3], W Daniel Stamer[1].
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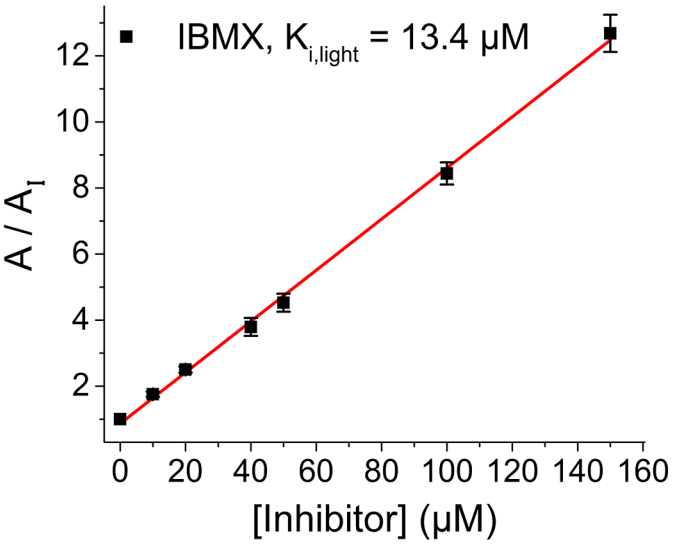
Purpose: Exfoliation glaucoma (XFG) is a clinically aggressive and genetically distinct form of glaucoma caused by extrafibullary material deposition in the trabecular meshwork, leading to increased intraocular pressure and death of retinal ganglion cells. Gene variants located in the promoter of a novel long non coding RNA (lncRNA), LOXL1-AS1 associate with XFG risk. Since lncRNAs can impact susceptibility to disease through alterations in gene expression, we investigated the downstream targets of LOXL1-AS1.
Methods: RNAseq was performed on cDNA derived from an immortalized lens epithelial cell line (B-3) after RNAi knockdown of LOXL1-AS1 or scrambled control to identify differentially expressed RNAs. To identify LOXL1-AS1 binding partners, we incubated biotinylated LOXL1-AS1 or control RNA with nuclear lysates followed by a pulldown using streptavidin beads. Proteins were resolved by SDS-PAGE, and identified through analysis of mass spectrometry data. Recombinant protein candidates and LOXL1-AS1 were incubated and immunoprecipitated *in vitro* to test direct binding and confirm mass spectrometry results.
Results: RNAseq identified 88 significantly altered RNAs, including 14 lncRNAs using parameters of logFC +/- 1.5 and $P < 0.0001$. Notably, several altered RNAs were filamentous/extracellular matrix proteins or involved in ECM homeostasis including INA, KRT14, and MMP9. To assess the mechanism by which LOXL1-AS1 alters gene expression, mass spectrometry revealed that LOXL1-AS1 interacts with the RNA processing proteins hnRNPL and ERF3A. Using protein/RNA binding studies, we demonstrated the direct binding of hnRNPL to LOXL1-AS1, through a 14 bp region in LOXL1-AS1. Conversely ERF3A, which was also identified by the mass spectrometry screen, bound to both LOXL1-AS1 and a complement RNAs indicating limited sequence specificity (false positive).
Conclusions: We observed that LOXL1-AS1 regulates a specific subset of target RNAs, and that this regulation may occur through interaction with hnRNPL. These findings strengthen the hypothesis that LOXL1-AS1 is a key player in XFG pathology and that targeting of the LOXL1-AS1/hnRNPL complex may provide novel therapeutic strategies for XFG.
Commercial Relationships: William M. Johnson, None; **Inas F. Aboobakar**, None; **Laura Finnegan**, None; **R Rand Allingham**, None; **Michael A. Hauser**, None; **W Daniel Stamer**, None

Program Number: 5591
Presentation Time: 12:30 PM–12:45 PM
Electrophysiological determination of inhibition constants for phosphodiesterase (PDE6) inhibitors
Teemu Turunen, Ari O. Koskelainen. Neuroscience and Biomedical Engineering, Aalto University, Espoo, Finland.
Purpose: PDE inhibitors are used to treat a variety of diseases but the lack of inhibitor specificity among different PDE families can inflict adverse side effects. The inhibition efficiencies of various PDE inhibitors towards photoreceptor PDE (PDE6) are mostly determined *in vitro* on enzymatically (trypsin) activated PDE6. In addition, the potencies of inhibitors towards trypsin-activated and thermally activated PDE6 have been shown to differ. However, methods to quantitatively study the inhibition potency of drugs towards light-activated PDE6 in natural surroundings are not available. This study proposes an *ex vivo* approach for quantitative determination of the inhibition constant towards light-activated PDE6 and thermally activated PDE6.
Methods: Transretinal electroretinography (ERG) and local ERG across the rod outer segments were used to record photoresponses from WT and GCAPs$^{-/-}$ dark-adapted mouse retinas at 37°C. Isolated retinas were superfused with solutions containing 2mM aspartate and 50μM $BaCl_2$ to separate the rod photoresponses. The inhibition constants for 3-isobutyl-1-methylxanthine (IBMX), sildenafil, and zaprinast towards light-activated PDE6 ($K_{i,light}$) were determined based on the decrease of phototransduction gain. In addition, a novel experimental paradigm was developed for electrophysiological determination of the inhibition constant of PDE inhibitors towards thermally activated PDE ($K_{i,thermal}$).
Results: The inhibition constants towards light-activated PDE6 ($K_{i,light}$) were 13.4 ± 0.7 μM for IBMX (n = 16 retinas, Fig. 1), 0.56 ± 0.09 μM for sildenafil (n = 4 retinas), and 0.97 ± 0.07 μM for zaprinast (n = 4 retinas). The $K_{i,thermal}$ of IBMX was 15.2 ± 0.2 μM (n=6 retinas, mean ± SEM).
Conclusions: This novel *ex vivo* approach allows a precise testing of the inhibition potency of drugs towards PDE6. Our results suggest that the K_i of IBMX has the same value for both the light-activated and the thermally activated forms of PDE6.



Determination of inhibition constant of IBMX towards light-activated PDE6.

Commercial Relationships: Teemu Turunen; **Ari O. Koskelainen**, None

Program Number: 5592
Presentation Time: 12:45 PM–1:00 PM

FIRST-IN-MAN PANCREATIC ISLET TRANSPLANTATION IN THE ANTERIOR CHAMBER OF THE HUMAN EYE

Nadine Gerber-Hollbach[1], Marc Donath[2], David Goldblum[1], Pascal W. Hasler[1], Beckey Trinh[2], Judith Siegenthaler[2], Matthias Hepprich[2], Thierry Berney[3, 4], Domenico Bosco[3], Juerg Steiger[2, 5], Michael Dickenmann[5], Christoph Henzen[6], Jeremy Meyer[4], Per-Olof Berggren[7]. [1]Ophthalmology, University Hospital of Basel, Basel, Switzerland; [2]Endocrinology, University Hospital of Basel, Basel, Switzerland; [3]Endocrinology, University Hospital of Geneva, Geneva, Switzerland; [4]Surgery, University Hospital of Geneva, Geneva, Switzerland; [5]Transplantation Immunology and Nephrology, University Hospital of Basel, Basel, Switzerland; [6]Medicine, Cantonal Hospital of Lucerne, Lucerne, Switzerland; [7]Karolinska Institute, Stockholm, Sweden.

Purpose: Diabetes type I results from the destruction of pancreatic islets due to autoimmune attack against the insulin-producing beta cells in the endocrine pancreas. While ongoing clinical trials have focused on islet transplantation into the hepatic portal system with some success, the search for alternative islet transplantation sites remains important. One location of interest is the anterior chamber of the eye. We report the feasibility and proof of concept of transplantation of allogenic pancreatic human islets for the first time into the anterior chamber of a human eye.

Methods: About 40'000 freshly prepared and isolated allogenic human islets (95% purity in 200 microliter) were transplanted into the anterior chamber of a legally blind diabetic eye of a c-peptide negative diabetic patient. Follow up over 3 months regarding clinical examination of the eye as well as fasting blood serum c-peptide and blood sugar levels were regularly determined.

Results: IOP increased up to 50 mm Hg the first night, but normalized over the next weeks. No inflammation or other ophthalmic complications were found otherwise in the injected eye. After mixed meal tolerance test c-peptide levels were measurable in the peripheral blood.

Conclusions: Our results proof that allogenic human pancreatic islet transplantation can be performed without causing permanent damage to the eye and that transplanted islets will survive and be functional after 3 months.

Commercial Relationships: Nadine Gerber-Hollbach; **Marc Donath**, None; **David Goldblum**, None; **Pascal W. Hasler**, None; **Beckey Trinh**, None; **Judith Siegenthaler**, None; **Matthias Hepprich**, None; **Thierry Berney**, None; **Domenico Bosco**, None; **Juerg Steiger**, None; **Michael Dickenmann**, None; **Christoph Henzen**, None; **Jeremy Meyer**, None; **Per-Olof Berggren**, None

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