ARVO 2014 Annual Meeting Abstracts

214 Meibomian Gland, Lacrimal Gland and Tear Film

Monday, May 05, 2014 8:30 AM–10:15 AM
S 331A-D Paper Session
Program #/Board #/Range: 1300–1306
Organizing Section: Cornea

Program Number: 1300
Presentation Time: 8:30 AM–8:45 AM
The Bioengineered Lacrimal Gland Using Cells Derived from Different Transgenic Mice: as a Novel Experimental Model for a Lacrimal Gland Biological Analysis
Masatoshi Hirayama, Tetsuya Kawakita, Shigeto Shimmura, Kazuo Tsubota. Ophthalmology, Keio Univ School of Medicine, Tokyo, Japan.

Purpose: The lacrimal gland, which develops through an epithelial-mesenchymal interaction during organ development, consists of acini, duct, myoepithelial cells and its peripheral tissues. Various types of cells are involved in the lacrimal gland development and regeneration process including tissue repair, however, it is difficult to analyze the origin and the kinetic of these cells. Here, we report a bioengineered lacrimal gland, which used cells derived from different transgenic mice as a model for analysis of the biology of the lacrimal gland development.

Methods: The care and handling of animals were performed in accordance with NIH guidelines. Protocols were approved by the Animal Care and Use Committee. We had successfully demonstrated the bioengineered lacrimal gland germ regeneration by organ germ method (ARVO2013). We regenerated the bioengineered lacrimal gland germ by using epithelial cells from transgenic mice and mesenchymal cells from the normal mice, and transplanted them to adult lacrimal gland defect model mouse. The development of the GFP-labelled bioengineered lacrimal gland, three-dimensional epithelial tissue morphology and the duct connection between the recipient and the bioengineered lacrimal gland were analyzed.

Results: The bioengineered lacrimal gland using cells from GFP transgenic mice was successfully developed at the transplantation site with duct connection between the bioengineered lacrimal excretory duct and the recipient’s lacrimal excretory duct. The epithelial cells of excretory lacrimal duct of the bioengineered gland, which were derived from DsRed-labelled epithelial cells, were successfully connected with the recipient’s lacrimal excretory duct histologically. The three dimensional morphological analysis revealed the GFP-labelled epithelial cells developed into branching morphogenesis, and it also revealed that myoepithelial cells were derived from epithelial cells during organ development.

Conclusions: We demonstrated that bioengineered lacrimal gland regeneration using cells derived from transgenic mice as a useful model to analyze the morphology and the developmental biology of the lacrimal gland. This model is expected to be applied for the analysis to clarify the biology of the lacrimal gland regeneration.

Commercial Relationships: Masatoshi Hirayama, None; Tetsuya Kawakita, None; Shigeto Shimmura, None; Kazuo Tsubota, None

Program Number: 1301
Presentation Time: 8:45 AM–9:00 AM
Meibomian Gland Dysfunction and Dyslipidemia: A Review
Puneet S. Braich1, Amarjot S. Mann1, Vikram Brar1, Christopher T. Leffler1, Vikram Lal2.
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Purpose: To investigate the relationship between meibomian gland dysfunction (MGD) and dyslipidemia [total cholesterol (TC) ≥ 200 mg/dL, low-density lipoprotein (LDL) ≥ 130 mg/dL, high-density lipoprotein (HDL) ≤ 40 mg/dL, triglycerides (TG) ≥ 150 mg/dL] in adult patients through a systematic review of the available data.

Methods: Data was gathered from three studies including our own. In all studies the participants had no prior diagnosis of dyslipidemia. Study-1, a case-control study of 60 symptomatic patients with MGD were compared to 63 age matched controls from the population. Study-2, a retrospective chart review of 93 patients with MGD were compared to 87 age matched comparisons from the same tertiary care center. Study-3, a retrospective chart review of 66 patients with MGD were compared to population data from the National Health and Nutrition Examination Survey (NHANES). All data was gathered from 2009-2012.

Results: In Study-1, dyslipidemia was found in 58.3% of cases and 6.3% of controls (P<0.01). Mean TC, LDL, and HDL were 210.8±4.4, 127.6±3.9, and 61.6±1.8 mg/dL, respectively, in cases and 162.9±3.1, 94.2±2.6, and 52.5±1.3 mg/dL, respectively, in controls. All differences were statistically significant (P<0.01).

In Study-2, dyslipidemia was found in 65.7% of cases and 13.3% controls (P<0.01). Mean TC and LDL were 217.4±8.4 and 122.4±5.7 mg/dL, respectively, in cases and 168.8±6.1, and 99.2±2.5 mg/dL, respectively, in controls. All differences were statistically significant (P<0.001). Logistic regression analysis revealed that in Study-1 and 2 patients with MGD were at 11-18% and 8-13% higher odds of having dyslipidemia.

In Study-3, patients with MGD had a higher prevalence of dyslipidemia by elevated TC (67.4% vs. 45.1%, P <0.05), when compared to population controls. There was a smaller number of MGD patients with low HDL (6.5% vs. 15.7%, P<0.05). Patients with MGD ended up having lower TG than controls (15.2% vs 33.1%, P<0.05). The incidence of increased LDL was not statistically significant.

Conclusions: Patients with MGD with no history of dyslipidemia may have undiscovered abnormal serum cholesterol levels compared to controls of similar age without MGD. Pending large scale studies showing similar results, MGD may become a sign of undiagnosed dyslipidemia and ophthalmologists will have a role in early detection of an important risk factor for cardiovascular disease.

Commercial Relationships: Puneet S. Braich, None; Amarjot S. Mann, None; Vikram Brar, None; Christopher T. Leffler, None; Vikram Lal, None

Program Number: 1302
Presentation Time: 9:00 AM–9:15 AM
DHA metabolism by human meibomian gland epithelial cells
Ulrike Hampel1,2, Todd W. Mitchell2, Simon H. Brown2, Peta Snikeris2, Garreis Fabian3, Friedrich P. Paulsen1, Mark D. Wilcox2.
1Medical faculty, Institute of Anatomy II, Erlangen, Germany; 2University of Wollongong, Illawara Health and Medical Research Institute and School of Health Sciences, Wollongong, NSW, Australia; 3University of New South Wales, School of Optometry and Vision Science, Sydney, NSW, Australia.

Purpose: To investigate the metabolism of the omega 3 fatty acid docosahexaenoic acid (DHA) by meibomian gland epithelial cells (MG cells) and production of one of its metabolites Resolvin D1 (RvD1).

Methods: MG cells were differentiated in DMEM containing EGF and DHA for up to 3 days. Optimal DHA concentration was assessed by measuring lipid droplet accumulation with Sudan III staining. Gene expression of cyclooxygenase-2 (COX-2) and 15- lipoxigenase (15-LOX) after DHA incubation was analyzed by real-time PCR. RvD1 concentrations of MG cell extracts were measured by enzyme
immune assay. Lipid cell extracts were analyzed by automated, chip-based nanofluidic analysis, ionization tandem mass spectrometry.

**Results:** Lipid droplet accumulation was increased by 100 µM DHA supplementation. COX-2 mRNA expression decreased by 31% compared to control during DHA supplementation after 3 days (p < 0.05). 15-LOX mRNA expression was reduced by 28% after three days of DHA incubation (p < 0.05). Both mRNA levels were not significantly decreased after 1 day incubation with DHA. The concentration of RvD1 was elevated 2-fold after DHA incubation (20.7 vs. 10.4 pg/mg total protein; p < 0.05). Total triglyceride (TAG) concentration was elevated during DHA supplementation.

**Conclusions:** DHA is esterified to TAG by MG cells. Furthermore, DHA supplementation supports anti-inflammatory effects by decreasing COX-2 expression and increasing the concentration of RvD1.

**Commercial Relationships:** Ulrike Hampel, Optima Pharmazeutische GmbH (F); Todd W. Mitchell, None; Simon H. Brown, None; Peta Snikeris, None; Garreis Fabian, None; Friedrich P. Paulsen, None; Mark D. Wilcox, None

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**Program Number:** 1304
**Presentation Time:** 9:30 AM–9:45 AM

**An exploratory study of lipid supplements on the tear film lipid layer of habitual soft contact lens wearers**

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**Purpose:** To determine the effect of exogenous lipid supplements on the clinical, functional and biochemical aspects of tear lipid layer in habitual contact lens wearers.

**Methods:** Ten participants were recruited to a randomized crossover trial which included a baseline visit with no intervention and visits after using an emulsion drop containing phosphatidylethanolamine (PE) (Systane Balance, Alcon) and a control saline drop (n=5), or a liposomal spray containing phosphatidylycholine (PC) (Tears again, BioRevue) and a saline spray as a control (n=5). Participants used the intervention/controls 3 times a day for two weeks with 24 hours washout period between the intervention and control visits. Visits occurred following 14 days of use of each intervention type or control after 8 hours of lens wear. All participants wore Ciba Vision, Air Optix® before starting each intervention. Ocular comfort scores, stability of the tear film (TBUT) and tear evaporation rate (TER) were assessed. Basal tears were collected into a glass microcapillary tube and analyzed by mass spectrometry. Comparison and associations between the variables were analyzed using Friedman’s ANOVA and Spearman correlation coefficient respectively.

**Results:** The mole % of PE in the total lipidome of those who used the emulsion drop was significantly (p=0.04) higher 0.94 ± 0.4% than those with saline drop 0.43 ± 0.3%. As the mole % of PE increased (R²=0.50 p=0.01) and TER significantly reduced (R²=0.20 p=0.03). There was a higher concentration of lysophosphatidylycholine (LPC) (mg/µl) in their tear lipidome when the liposomal spray was used and this resulted in an increased tear evaporation rate (R²=0.50 p=0.03) and a shorter but not statistically significantly TBUT (R²=0.10 p=0.06). The increased mole % of lysophospholipids (LPC + LPE) was associated with a decreased ocular comfort (R²=0.10 p=0.02) during lens wear.

**Conclusions:** Compared with placebo, the exogenous phospholipid emulsion drop significantly changed the mole ratio of PE in the tear lipidome of habitual contact lens wearers and improved their tear film stability and tear evaporation rate. Interestingly, an adverse effect of lysophospholipids on the tear evaporation rate and ocular comfort.
was observed. However, these observations needed to be confirmed in a larger population.

**Commercial Relationships:** Athira Rohit, None; Mark D. Wilcox, None; Todd W. Mitchell, None; Fiona Stapleton, None

**Clinical Trial:** ACTRN12613001323718

**Program Number:** 1305
**Presentation Time:** 9:45 AM–10:00 AM

**Surfactant Protein-H (SFTA3), a novel regulatory surfactant protein at the ocular surface and in the tear film**

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**Purpose:** To detect and characterize a novel surfactant protein at the ocular surface. Surfactant proteins (SP) are well known from human lung. These proteins assist the formation of a monolayer of surface-active phospholipids at the liquid-air interface of the alveolar lining, play a major role in lowering the surface tension of interfaces, and have functions in innate and adaptive immune defense. During recent years it became obvious that SPs are also part of other tissues and fluids such as saliva, kidney or also tear fluid. Recently, a putative new surfactant protein (SFTA3 or SP-H) was identified, which has no sequence or structural identity to the already know surfactant proteins.

**Methods:** In this work, computational chemistry and molecular-biological methods were combined to localize and characterize SP-H. With the help of a protein structure model, specific antibodies were obtained which allowed the detection of SP-H. SP-H expression was analyzed by Western-blot analysis, ELISA as well as immunohistochemistry in different tissues of the ocular surface and in tear fluid. The activation and regulation of SP-H transcription was studied in human cornea (HCE) and conjunctiva (HCJE) epithelial cell lines after incubation with ocular pathogens by real-time RT-PCR. Furthermore, the protein concentration of SP-H was measured by ELISA in tears from patients suffering from dry eye disease (DED) in comparison to healthy volunteers.

**Results:** The localization of SP-H in cornea, eye lid, lacrimal gland and meibomian gland, sequence based prediction tools for posttranslational modifications and molecular dynamic simulations revealed that SP-H has physicochemical properties similar to the already known surfactant proteins B and C. This includes also the possibility of interactions with lipid systems and with that, a potential surface-regulatory feature of SP-H. Stimulation experiments in HCE and HCJE with pathogens showed an increased expression of SP-H. In tears from DES patients SP-H concentration was increased compared to controls.

**Conclusions:** In conclusion, the results indicate SP-H as a novel surfactant protein of the ocular surface and tear film which represents an until now unknown surfactant protein class. SP-H seems to have surface tension-regulatory features and is upregulated under experimental inflammatory conditions and in cases of DED indicating further immune modulatory functions.

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**Program Number:** 1306
**Presentation Time:** 10:00 AM–10:15 AM

**Alteration of Galectin-3 in Tears of Dry Eye Patients**

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**Purpose:** Galectin-3 is a multimeric carbohydrate-binding protein known to be upregulated under several pathological conditions, such as inflammation and cancer. Proteolytic cleavage of galectin-3 by matrix metalloproteinases (MMPs) has been associated with abrogation of the biological properties of the lectin and with the progression of the disease. Here, we investigated the secretion and cleavage of galectin-3 in tears of dry eye patients.

**Methods:** Tear fluid and conjunctival impression cytology specimens were collected from normal subjects (n=11) and patients with non-Sjögren’s dry eye (n=20). Galectin-3 content in tears was analyzed by quantitative Western blot, using recombinant galectin-3 protein as the calibration standard. The relative expression of MMP9 mRNA in conjunctival impression cytology specimens was evaluated using quantitative PCR (qPCR). Galectin-3 cleavage and inhibition assays were performed in vitro using activated recombinant MMP9.

**Results:** The level of galectin-3 was significantly higher in tears of dry eye patients (Ave. 0.38 ng/µg total protein, range 0.04-1.36) compared to controls (Ave. 0.12 ng/µg total protein, range 0.00-0.41) (p<0.01). By Western blot, all tear samples from normal subjects were characterized by the presence of a single band corresponding to full-length galectin-3, whereas 50% of dry eye patients contained both full-length and cleaved galectin-3. Analyses of conjunctival epithelium by qPCR revealed increased MMP9 mRNA expression in dry eye patients. Importantly, we demonstrated that active MMP9 can cleave full-length galectin-3 from recombinant origin and from tear fluid. These effects were abrogated by use of the broad-spectrum MMP inhibitor GM6001.

**Conclusions:** Tear fluid of patients with dry eye contains increased levels of galectin-3. Proteolytic cleavage of galectin-3 by MMP9 may lead to ocular surface barrier dysfunction and be potentially used to monitor inflammation in dry eye disease.

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