Purpose: To study the corneal nerve morphology, electrical nerve activity and epithelial wound healing in young and adult guinea pigs.

Methods: Young (1-3 months) and adult (7-9 months) guinea pigs of both sexes were used in compliance with the ARVO Statement on the Use of Animals.

Nerve morphology: Dissected corneas were fixed with osmium tetroxide and lead citrate. Whole-mount corneas were stained with neuronal class III β-tubulin antibody. Nerve density was calculated from camera lucida drawings and confocal microscopy images.

Wound healing: 2 mm-diameter corneal epithelial debridments were performed with a drop of 2% acetic acid. Fluorescein-stained lesions were photographed regularly until complete closure. Images were analyzed with image processing software, and epithelial migration rate (EMR) and estimated time of healing (ETH) were calculated.

Nerve recording: Whole eyes or isolated corneas were superfused with physiological saline at 34°C. Electrical activity was recorded using conventional electrophysiological equipment. The response to thermal (changing the temperature of the solution down to 20°C or up to 50°C), mechanical (calibrated von Frey hairs) and chemical stimulation (30s gas jets of CO2 applied to the corneal surface) was explored. The characteristics of the spontaneous and stimulus-evoked activity were analyzed.

Basal tear rate was also measured in young and adult guinea pigs using phenol red threads.

Results: Density of subbasal nerves was significantly lower in adult animals than in young animals, while their length increased significantly. Also subbasal nerves leashes appeared less branched and the number of epithelial nerve terminals was lower in adult guinea pigs. EMR was slower and ETH was significantly increased in adult animals compared to young. However, no significant differences were observed between spontaneous or stimulus-evoked activity of the different types of corneal sensory receptors recorded from young and adult animals. Basal tear secretion was similar in young and adult animals.

Conclusions: The corneal nerve architecture changes with time in the guinea pig, exhibiting a reduction in the subbasal and epithelial nerve density, which might lead to a neurotrophic slowdown of epithelial wound healing in adult animals. In spite of that, the characteristics of the spontaneous and stimulus-evoked activity of corneal nerve activity are similar in young and adult animals.

Commercial Relationships: M Carmen Acosta, None; Kamila Mizerska, None; Carolina Luna, None; Susana Quirce, None; David Berbel, None; Julio Sesma, None; Nicolas Cuenca, None; Carlos Belmonte, None; Juana Gallar, None


Program Number: 3645 Poster Board Number: A0159
Presentation Time: 3:45 PM–5:30 PM

In vivo Functional Characterization of Trigeminal Neurons Innervating the Eye and Periocular Tissues

Juana Gallar, Baldemar Santiago, M Carmen Acosta, Carlos Belmonte. Instituto de Neurociencias, Universidad Miguel Hernandez-CSIC, San Juan de Alicante, Spain.

Purpose: The aim of this work was to study in vivo the firing characteristics of the different functional classes of trigeminal primary sensory neurons innervating cornea, sclera, bulbar and palpebral conjunctiva, and eyelids.

Methods: Anesthetized Wistar male rats (300±25g) were placed in a stereotaxic frame and electrical activity of trigeminal ganglion (TG) neurons was recorded extracellularly with a tungsten electrode (2MΩ) introduced into the TG. Heart rate, expiratory CO2, SpO2 and rectal temperature were monitored. Electrical signals were amplified (1000x), filtered (300Hz, 10KHz) and recorded at 20KHz using an ADC interface and software for off-line analysis. Mechanical stimulation of the ipsilateral cornea and surrounding tissue was performed using a fine brush and calibrated von Frey filaments, mechanical threshold was measured and receptive field (RF) mapped. CO2 gas puffs and 20µl drops of 100µM menthol applied onto the ocular surface were used for chemical stimulation. Additionally, the corneal surface was subjected to variable conditions of humidity, and the receptive fields of the units were stimulated electrically (0.1-2ms, 15V) to measure the conduction velocity of the afferent fiber.

Results: Neurons innervating the superior eyelid (11), the inferior eyelid (3), the cornea (5) and the conjunctiva (7) were identified. Neurons with RFs covering the eyelids were classified as mecanororeceptive by their response to punctate, stretching and pulling mechanical stimuli; their mechanical threshold varied from 0.07 to 1.56 mN in the hairy and shaved skin respectively. Neurons with corneal and conjunctival RFs showed ongoing activity, increasing their firing to decreased temperature, augmented evaporation, CO2 puffs and menthol drops, being classified as cold thermosensory neurons. Part of the corneal and conjunctival neurons responded only to punctate stimuli (threshold around 0.07mN).

Conclusions: These results proved the feasibility of using high impedance tungsten electrodes for stable, long-lasting recordings in vivo of the different functional classes of TG neurons innervating the ocular surface, thus opening the possibility of analyzing their activity under different experimental conditions.

Commercial Relationships: Juana Gallar, None; Baldemar Santiago, None; M Carmen Acosta, None; Carlos Belmonte, None

Program Number: 3646 Poster Board Number: A0160
Presentation Time: 3:45 PM–5:30 PM

Dry-eye-like Symptoms without Dissociated Signs Implies Corneal Neuropathy: An In Vivo Confocal Microscopy Study

Yimin Li, JianJiang Xu, Jiaxu Hong, Qihua Le. Eye & ENT Hospital, Fudan University, Shanghai, China.

Purpose: To characterize the symptoms, signs and corneal innervation of patients with dry-eye-like symptoms but without dissociated signs, with the aid of laser in vivo confocal microscopy.

Methods: Twenty-five eyes of 25 suspected corneal neuropathy patients, 24 eyes of 24 age- and sex-matched mild to moderate non-Sjögren’s syndrome dry eye patients (MMDE), and 24 healthy non-Sjögren’s syndrome dry eye patients (MMDE).

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controls were recruited to the study. Ocular surface disease index (OSDI), visual analog scales (VAS), and Hospital Anxiety and Depression Scale (HADS) were used to assess subjective symptoms or psychological evaluation. Slit lamp examination, Schirmer I test (SI), tear breakup time (TBUT), corneal fluorescein staining and laser in vivo confocal microscopy were conducted. The mechanical corneal sensitivity was measured using Cochet-Bonnet esthesiometer.

**Results:** The mean OSDI and VAS scores were higher in neuropathy and MMDE patients (P<0.001). The scores of both HADS anxiety and depression subscales among all the subjects were in normal range. The values of SI (P=0.013) and TBUT (P<0.001) were lower in MMDE patients. The average corneal sensation thresholds for neuropathy patients was lower (P=0.003). The mean corneal nerve density, nerve counts, and LC density, were significantly higher in neuropathy group. The LC density, gender, average corneal sensitivity, and nerve density were independent factors for the OSDI or VAS scores.

**Conclusions:** Without desiccated signs, dry-eye-like symptoms could be caused only by corneal nerve alteration, immune reaction, and hypersensitivity, especially among female gender. Corneal neuropathy should be recognized instead of misclassified into suspected dry eye disease.

**Purpose:** Understanding the ocular surface inputs for blinking and sensation is important for dry eye and related conditions, particularly since the ocular surface input for blinking is controversial. The purpose of this study was to determine how increasing ocular surface stimulation affected blinking and sensation, while controlling task performance.

**Methods:** Ten healthy subjects played video games to control their concentration, while seated behind a slit lamp biomicroscope fitted with a custom device generating air flow (AF) toward the central cornea. After estimating the AF threshold resulting in increased blinking, 6 levels of stimuli were randomly presented 3 times each and subjects used visual analog scales to record their sensory responses. The interblink interval (IBI) and the AF were recorded simultaneously and MATLAB programs determined IBI, sensory response and corresponding AF for each trial. A blink increase threshold (BIT), the AF associated with a statistically significant decrease in IBI, was calculated for each subject.

**Results:** Blinking was highly variable between subjects at baseline, with a mean IBI (±SD) of 5.69±3.92sec. The BIT (±SD) was 98±26 ml/min air flow with IBI=2.84±1.19sec (permutation test, p<0.001). After log transformation, there was a significant linear function between increasing AF and decreasing IBI within each subject (-0.859± Pearson’s r= 0.987, p<0.05). The IBI was correlated with watery, discomfort and cooling ratings (Pearson’s r= -0.737, -0.606 and -0.632, p<0.001).

**Conclusions:** In normals, ocular surface stimulation increases blinking, following a dose response relationship. Blinking was correlated with ocular surface sensation, presumably due to their common ocular surface input. The BIT, a novel metric introduced here, may provide additional endpoints for dry eye or other ocular surface studies.

**Commercial Relationships:** Ziwei Wu, None; Carolyn G. Begley, None; Ping Situ, None; Trefford L. Simpson, None

**Program Number:** 3648 Poster Board Number: A0162
**Presentation Time:** 3:45 PM–5:30 PM

**Effects of Corneal Nerve Density on the Response to Treatment in Dry Eye Disease**

**Ahmad Kheirkhah, Thomas H. Dohman, Francisco Amparo, Michael A. Arnoldner, Yureeda Qazi, Arisia Jamali, Pedram Hanrah, Reza Dana. Ophthalmology, Massachusetts Eye and Ear Infirmary, Boston, MA.**

**Purpose:** Corneal nerves, which play a critical role in maintaining corneal epithelial health, may be diminished in dry eye disease (DED). We hypothesized that variability in patients’ responses to dry eye therapy may, in part, be due to different levels of corneal nerve density. Thus, this study aimed to evaluate whether levels of corneal subbasal nerve fiber length (SNFL) before DED treatment could prognosticate the level of improvement in signs and symptoms after treatment.

**Methods:** This double-masked clinical trial included 37 patients with DED and 27 age-matched controls. Patients with DED were randomized to receive either loteprednol 0.5% suspension (Lotemax®) or artificial tears (AT), both twice daily for 4 weeks. At baseline, in vivo confocal microscopy (Heidelberg Retina Tomograph 3/Rostock Cornea Module) of the central cornea was performed in both eyes of all subjects. Corneal SNFL was measured by two baseline, in vivo confocal microscopy (Heidelberg Retina Tomograph 3/Rostock Cornea Module) of the central cornea was performed in both eyes of all subjects. Corneal SNFL was measured by two
all therapeutic groups and compared between subgroups with low and near-normal SNFL.

Results: In patients with DED, SNFL (17.07 ± 6.62 mm/mm²) was significantly lower than in controls (23.33 ± 3.25, P=0.001). In the loteprednol group, although no significant improvement in any sign or symptom was noted in patients with low SNFL (<16.84 mm/mm²), subjects with near-normal SNFL (≥16.84 mm/mm²) showed significant improvement in both symptoms (P=0.04) and corneal fluorescein staining (CFS, P=0.008). Among the AT group, while cases with low SNFL (<16.84 mm/mm²) showed no significant change in any sign or symptom, subjects with near-normal SNFL (≥16.84 mm/mm²) demonstrated significant improvement in symptoms (P=0.01) and CFS (P=0.006).

Conclusions: Significant improvement of clinical signs and symptoms after DED treatment was evident only in the group with near-normal corneal SNFL. Consideration of corneal subbasal nerve density may thus assist in explaining the variability of patients’ responses to DED therapy. In addition, subject segmentation based on corneal nerve density may assist in improving predictability of response to therapy in DED clinical trials.

Commercial Relationships: Ahmad Kheirkhah, None; Thomas H. Dohlman, None; Francisco Amparo, None; Michael A. Arnoldner, None; Yureeda Qazi, None; Arsia Jamali, None; Pedram Hamrah, Massachusetts Eye and Ear Infirmary, Application number: PCT/US2013/027181 (P); Reza Dana, Bausch & Lomb (C)

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Clinical Trial: NCT01456780

Program Number: 3649 Poster Board Number: A0163

Presentation Time: 3:45 PM–5:30 PM

Age-Related Corneal Sensitivity assessed with a modified BHVI-Belmonte Aesthesiometer: a preliminary study

Alex Gonzalez1, Klaus Ehrmann2, Cornelis Rowaan1, William Lee1, Mariela C. Aguilar1, Allison L. McClellan3, Nabeel M. Shalabi1, 3, Anat Galor1, 3, Jean-Marie A. Parel1, 2. 1Ophthalmic Biophysics Center, Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami, FL; 2Vision Cooperative Research Centre, Brien Holden Vision Institute, UNSW, Sydney, NSW, Australia; 3VA Medical Center, Miami, FL.

Purpose: The BHVI-Belmonte aesthesiometer (Br J Ophthalmol 2004;88:1547–1551), an air/CO2 jet instrument developed to allow stimulation of the ocular surface using distinct mechanical, chemical, and thermal stimuli, was modified to improve the precision of stimulus localization, distance between the ocular surface and the instrument probe, and stimulus delivery.

Methods: To improve stimulus localization, probe distance and stimulus delivery, the aesthesiometer was modified with the addition of dual cameras (1MP, 15fps) focused on the OS and OD corneal surface, blue LED illumination for fluorescein staining, and the original incandescent illumination was replaced with a user dimmable LED (6000mcd, 5900K, Super Bright LEDs Inc, St Louis, MO). Fixation lights mounted on the instrument housing enabling simple adjustment to the stimulus location were enhanced by making them user selectable in 4 regions (corneal center, mid-periphery, limbus and anterior conjunctiva) in all 4 quadrants. A PC-based graphical user interface (National Instruments, Austin, TX) was designed to record the video and flow volume data, track the interval between stimuli, and calculate the flow volume rate for succeeding stimuli based on subject’s sensation response utilizing an unequal staircase algorithm. Measurements were collected under a VA IRB on 6 healthy subjects, 2 males, 4 females (age 22-63). Sessions were held at the same time of day, each subject was measured 2 times with an interval between data collection of 32 days ±15.

Results: Subjects were found to have a mean predicted corneal sensitivity threshold of 47.9±14 (ml/min), the Cronbach’s alpha was 0.75, indicating good reliability and showed positive statistically significant correlation with age (r = 0.933, P=0.002).

Conclusions: Modifications to the BHVI-Belmonte Aesthesiometer enhanced the precision of stimulus localization, distance between the ocular surface and the instrument probe, and stimulus delivery and the preliminary data indicate a correlation between age and corneal sensitivity.
Age Related Influence on Corneal Sensitivity.

Commercial Relationships: Alex Gonzalez, None; Klaus Ehrmann, None; Cornelis Rowaan, None; William Lee, None; Mariele C. Aguilar, None; Allison L. McClelland, None; Nabeel M. Shalabi, None; Anat Galor, None; Jean-Marie A. Parel, None
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Program Number: 3650 Poster Board Number: A0164
Presentation Time: 3:45 PM–5:30 PM
Loss of Muc16 induces activates Stat3 signal and IL-6 expression in conjunctiva and secondarily affects corneal wound healing in mice
Shizuya Saika1, Kumi Shiba1, Yuka Okada1, Masayasu Miyajima2, Richard R. Behringer3, Osamu Yamanaka1. 1Ophthalmology, Wakayama Medical University, Wakayama, Japan; 2The Laboratory Animal Center, Wakayama Medical University, Wakayama, Japan; 3Program in Genes and Development, The University of Texas Graduate School of Biomedical Sciences, Houston, TX; 4Genetics, The University of Texas Graduate School of Biomedical Sciences, Houston, TX.
Purpose: To investigate the inflammatory process in conjunctiva of a Muc16-null (KO) mouse and affects of the loss of Muc16 on corneal epithelium and keratocytes post-epithelial debridement.
Methods: KO mice (n = 40) and C57/BL6 (wild type, WT) mice (n = 40) were used. Expression of phospho-Stat3, AP-1 components, interleukin 6 (IL-6) and tumor necrosis factor alpha in cornea and conjunctiva was examined in WTand KO mice. Epithelial cell proliferation was studied by using BrdU labeling. Finally, wound healing of a round defect (diameter: 2.0 mm) in corneal epithelium and keratocytes post-epithelial debridement. Keratocyte phenotype and macrophage invasion in the stroma were evaluated after epithelium was recovered.
Results: Lacking Muc16 activated Stat3 signal as well as upregulated expression of IL-6 mRNA in conjunctiva. Loss of Muc16 accelerated wound healing of corneal epithelium. The incidence of myofibroblast appearance and macrophage invasion were more marked in KO stroma as compared with WT stroma after epithelium repair.
Conclusions: Lacking Muc16 secondarily affects the homeostasis of corneal epithelium and stroma. The mechanism might include upregulation of inflammatory signaling cascade, i. e., Stat3 signal, and IL-6 expression in KO conjunctiva.
Commercial Relationships: Shizuya Saika, None; Kumi Shiba, None; Yuka Okada, None; Masayasu Miyajima, None; Richard R. Behringer, None; Osamu Yamanaka, None

Program Number: 3651 Poster Board Number: A0165
Presentation Time: 3:45 PM–5:30 PM
Effect of Chondrocyte-derived Extracellular Matrix on Dry Eye Mouse Model
Chea Eun Kim1, Ji Hyun Lee1, JaeWook Yang1, 2. 1Ocular Neovascular Disease Research C, Inje University Busan Paik Hospital, Busan, Republic of Korea; 2Department of Ophthalmology, Inje University College of Medicine, Busan, Republic of Korea.
Purpose: To investigate the effect of chondrocyte-derived extracellular matrix (CDECM) on the change of cornea and conjunctiva in dry eye mouse model.
Methods: This study was conducted in accordance with the Guidelines for Animal Experiments approved by Inje University College of Medicine (No.; 2012-053) and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Experimental dry eye was created in 12-to 16-week-old NOD.B10.H2b mice by subcutaneous injection of scopolamine with exposure to an air draft for 10 days. Tear volume and corneal smoothness were measured at 0, 10 days after removal of desiccating stress, and instillation of phosphate buffered saline (PBS group) or CDECM (CDECM group) after the removal of desiccating stress for 3, 5, 7 and 10 days. Cornea and conjunctiva were hematoxylin and eosin (H&E) staining and periodic acid Schiff (PAS)-stained sections. Expression of inflammation was detected by immunohistochemistry.
Results: Instillation of CDECM after the removal of desiccating stress, the tear production compared to the desiccating stress decreased to 3.3% (P < 0.05) was increased to 94.9% (P < 0.05) in the 10 days. However, instillation of PBS for 10 days after the removal of desiccating stress, tear production compared to the desiccating stress was increased to 21.1% (P < 0.05). In CDECM group, the corneal smoothness improved at 3-10 days, and increased at conjunctival goblet cells and anti-inflammation effects, and decreased at detach of corneal epithelial cells. In contrast, in PBS group was hardly recovered.
Conclusions: Instillation of CDECM after the removal of desiccating stress in experimental dry eye model, tear volume, corneal smoothness, conjunctival goblet cells, corneal epithelial cells detach, and anti-inflammation effects recovered within 10, whereas they remained unchanged in PBS group. These observations suggest that CDECM were effectiveness of anti-inflammatory improvement on the cornea and conjunctiva in experimental dry eye model.
Commercial Relationships: Chea Eun Kim, None; Ji Hyun Lee, None; JaeWook Yang, None
Support: Korea Healthcare Technology R&D project, Ministry of Health and Welfare (Grant HI12C0005)

Program Number: 3652 Poster Board Number: A0166
Presentation Time: 3:45 PM–5:30 PM
Effectiveness of Topical AICA-Ribonucleotide in a Mouse Model of Experimental Dry Eye
Mi Sun Sang1, Zhenri Li1, Jee Myung Yang1, Ji Suk Choi1, InCheon You1, Kyung Chul Yoon1. 1Ophthalmology, Chonnam National University Medical School and Hospital, Gwangju, Republic of Korea; 2Ophthalmology, Chonbuk National University Medical School and Hospital, Jeonju, Republic of Korea.
Purpose: To investigate the efficacy of a topical AICA-Ribonucleotide (AICAR) in a mouse model of experimental dry eye (EDE)
Methods: Eye drops consisting of 0.001% and 0.01% AICAR, or 0.05% cyclosporin (CsA) were applied in EDE. Tear volume, tear film break-up time (TBU), and corneal fluorescein staining scores were measured at 10 days after treatment. Levels of interleukin (IL)-β, IL-6, tumor necrosis factor (TNF)-α, interferon (IFN)-γ,
monokine induced by interferon-γ (MIG) and Interferon gamma-induced protein 10 (IP-10) were measured in the conjunctiva using a multiplex immunobead assay. Periodic acid-Schiff staining, immunofluorescence staining and flow cytometry were also performed.

Results: Mice treated with 0.01% AICAR showed a significant improvement in tear volume, TBUT and corneal fluorescein staining scores compared with the EDE control and other treatment groups. A significant decrease in the levels of IL-1β, IL-6, TNF-α, IFN-γ, MIG and IP-10 and the number of CD11b+ and CD4+CXCR3+ cells and an increase in goblet cell density was observed in the 0.01% AICAR-treated group, compared with the control and other treatment groups. 0.05% Csa also led to an improvement in the tear and corneal signs and inflammatory molecules compared with the control. However, there were no significant differences in all parameters between the 0.001% AICAR and EDE control groups.

Conclusions: Topical application of 0.01% AICAR could markedly improve clinical signs and decrease inflammation in the ocular surface of EDE, suggesting that AICAR eye drops may be used as a therapeutic agent for dry eye disease.

Commercial Relationships: Mi Sun Sung, None; Zhengri Li, None; Jee Myung Yang, None; Ji Suk Choi, None; In-Cheon You, None; Kyung Chul Yoon, None

Program Number: 3653 Poster Board Number: A0167
Presentation Time: 3:45 PM–5:30 PM
Regulatory T cell mediated suppression of dendritic cells in a mouse model of dry eye
Katherine S. Held1, Chris S. Schaumburg1, Jianping Gao1, Julio Nieves1, Euikyon Oh1, Larry A. Wheeler1, Virginia L. Calder2, None; Euikyon Oh, Allergan, Inc. (E); Larry A. Wheeler, Allergan, Inc. (E); Virgina L. Calder, Allergan, Inc. (C); Jerry Y. Niederkorn, Allergan, Inc. (C); Stephen C. Pfugfelder, Allergan, Inc. (C); Michael E. Stern, Allergan, Inc. (E)

Purpose: To determine the anti-inflammatory effect of two polyphenols, quercetin (QCT) and resveratrol (RES), and a combination (QCT+RES) in a mouse model of dry eye.

Methods: Dry eye was induced in female C57BL/6 mice exposing them to a desiccating stress (DS): 23°C, 20% relative humidity, constant airflow and subcutaneous scopolamine administration 0.1 mg/d for 10 days. QCT, RES, QCT+RES or vehicle were applied topically 3 times a day starting day -1. Non exposure and untreated mice were used as controls. CD4+ T cells isolated from DS and control mice were transferred to nude C57BL/6 recipient mice and were evaluated after 3 days of transfer. Tear production, conjunctival goblet cell density, CD4+ T cell infiltration in conjunctiva and cytokine production in tears were evaluated in DS, control and recipient mice at the end of the assay. Corneal fluorescein staining (CFS) was performed in control and DS mice. Cytokine production in tears was additionally evaluated at day 6 in DS mice.

Results: DS altered the ocular surface increasing CFS after 10 days. This effect was decreased by QCT (P<0.001) and QCT+RES (P<0.05) treatments, compared to vehicle. DS provoked an increase in tear levels of IL-1α and RANTES after 6 days of exposure. QCT, RES and QCT+RES treatment significantly decreased IL-1α levels in tears compared to vehicle, (P<0.05, 0.01 and 0.01, respectively). Neither QCT, RES or the combination had any effect on tear production, goblet cell density and CD4+ T cell infiltration in DS mice. However, a significant increase in tear production (P<0.01) was found in recipients of QCT+RES treated mice, compared to recipients of vehicle. Moreover, CD4+ T cell infiltration in conjunctiva was found to be significantly lower (P<0.05) in recipients of RES treated mice, compared to that observed in recipients of vehicle mice. Cytokine production in tears and goblet cell density in recipient mice were not affected by polyphenols treatments.

Conclusions: Topical treatment with QCT, RES or QCT+RES reduced several inflammatory changes and clinical signs in an experimental mouse dry eye model, decreasing IL-1α production in tears and corneal staining in DS mice, and tear production and CD4+ T cell conjunctival infiltration in recipient mice. These results suggest that the topical application of both compounds could be used for the treatment of dry eye.
### Environmental Stress Conditions in the Nrf2(-/-) Mouse

**Purpose:** The current study revealed that Nrf2 is involved in the pathogenesis of ocular surface damage and decreased tear secretion under environmental stress conditions.

**Methods:** Thirty week old C57/B6 wild type mice (wt) and Nrf2 (-/-) mice (6 mice in each group) were used for evaluations of environmental stress conditions on the ocular surface and tear secretion.

**Results:** After stress exposure, tear secretion was significantly decreased in both wild type (p=0.0015) and Nrf2 (-/-) mice (p=0.0043). Mean percentage of tear secretion reduction in the Nrf2 (-/-) mice (62.8±16.6%) was significantly higher than the wild type mice (17.2±13.8%) (p=0.017). Tear film break-up time after stress in the Nrf2 (-/-) mice was significantly shorter than the wild type mice after exposure to the stress conditions (p=0.036). There were no differences in the mean fluorescein staining scores after stress exposure between the Nrf2 (-/-) mice (3.5±1.3 points) and the wild type mice (2.8±1.7 points) (p=0.158). The mean Rose Bengal score after stress exposure in the Nrf2 (-/-) mice (3.4±0.9 points) was significantly higher than the wild type mice (2.3±0.8 points) (p=0.01).

**Conclusions:** The current study revealed that Nrf2 is involved in the pathogenesis of ocular surface damage and decreased tear secretion under environmental stress conditions.

### Commercial Relationships:
- Antonio Abengózar-Vela, University of Valladolid (P); Chris S. Schaumburg, Allergan Inc. (E); Michael E. Stern, Allergan Inc. (E); Virginia L. Calder, Allergan Inc. (C); Margarita Calonge, Allergan Inc. (C), University of Valladolid (P); Amalia Enríquez-de-Salamanca, University of Valladolid (P); María-Jesus J. Gonzalez, University of Valladolid (P)
- Support: Antonio Abengózar-Vela was supported by “ayudas para estancias breves en el desarrollo de tesis doctorales” - University of Valladolid

### Program Number: 3655 Poster Board Number: A0169
### Presentation Time: 3:45 PM–5:30 PM

### Effects of Mesenchymal Stem/Stromal Cells in T cell-mediated Dry Eye Model in Mice

**Purpose:** To investigate the effects of mesenchymal stem/stromal cells (MSCs) in T cell-mediated dry eye model in mice that was induced by concanavalin A (ConA) into lacrimal glands.

**Methods:** Either phosphate buffered saline (PBS) or Con A (10mg/ml) was injected into lacrimal glands in mice. Immediately after ConA injection, balanced salt solution (BSS), 1X10³ MSCs, or 1X10⁵ MSCs were injected periocularly near lacrimal glands. One week later, tear production was measured by phenol red thread test, and levels of interleukin (IL) -2 and interferon (IFN)-γ were evaluated in the lacrimal glands and the ocular surface using real time RT-PCR. Infiltration of inflammatory cells and damage to the lacrimal glands were evaluated by histopathology, and ocular surface goblet cell count was performed. Also, infiltration of immune cells in lacrimal glands was analyzed by immunohistochemistry and flow cytometry.

**Results:** Con A injection induced T cell-mediated dry eye in mice by causing T cell infiltration, increasing pro-inflammatory cytokine expression. Also tear production was significantly decreased (p=0.0006) and goblet cell count was reduced (p=0.02). A Both 1X10³ MSCs and 1X10⁵ MSCs significantly improved tear production (vs. ConA+BSS group, p=0.001 and p=0.0006, respectively). Also, MSCs significantly decreased the levels of IL-2 and IFN-γ in the ocular surface and lacrimal glands. Similarly, the numbers of IFN-γ-expressing CD4⁺ cells in lacrimal glands were significantly lower in MSC-treated groups compared to Con A+BSS group (p=0.008 for 1X10³ MSCs, p=0.004 for 1X10⁵ MSCs).

**Conclusions:** Periorbital injection of MSCs reduce inflammation of the ocular surface and lacrimal glands, and improve tear production in Con A-induced dry eye model in mice.
Tear production was significantly reduced after ConA injection, but both 1X10^3 MSCs and 1X10^5 MSCs significantly improved tear production. *** p<0.005

The expression of IL-2 and IFN-\(\gamma\) in the lacrimal glands were significantly increased after ConA injection, but reduced with MSCs injection.

* p<0.05, ** p<0.01

Commercial Relationships: Min Joung Lee, None; Ah Young Ko, None; Jung Hwa Ko, None; Mee Kum Kim, None; Won Ryang, None

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Program Number: 3657 Poster Board Number: A0171
Presentation Time: 3:45 PM–5:30 PM
Impact of the Host Microbiota on the Development of Murine Desiccating Stress-Induced Dry Eye Disease

Chris S. Schaumburg,1 Katherine S. Held,1 Annie M. Ratanapinta1, Madhuri Paul1, Larry A. Wheeler1, Margarita Calonge2, Jerry Y. Niederkorn1, Stephen C. Pflugfelder4, Michael E. Stern1.

1Biological Sciences, Allergan, Irvine, CA; 2Institute for Applied OphthalmoBiology (IOBA), University of Valladolid, Valladolid, Spain; 3Ophthalmology, UT Southwestern Medical Center, Dallas, TX; 4Ocular Surface Center, Cullen Eye Institute, OphthalmoBiology, Baylor College of Medicine, Houston, TX.

Purpose: Recently, alterations in the host microbiota have been shown to impact the development of various autoimmune diseases. To begin to understand the contribution of commensal enteric bacteria on the development of Dry Eye disease, C57BL/6 mice were treated with a broad spectrum antibiotic cocktail, and then exposed to desiccating stress (DS) to induce experimental Dry Eye disease.

Methods: Dry Eye disease was induced by exposing female C57BL/6 mice to desiccating stress (DS: subcutaneous scopolamine, 0.1mg/day; humidity 20%; sustained airflow). Three weeks prior to inducing Dry Eye, commensal flora were depleted by treating mice with an antibiotic cocktail consisting of the antifungal amphotericin-B (1 mg/kg, oral gavage BID), ampicillin (1 mg/ml, ad libitum via drinking water), and oral gavage of vancomycin (50 mg/kg), neomycin (100 mg/kg), metronidazol (100 mg/kg) and amphotericin-B (1 mg/kg) administered every 12 hours. To ensure the antibiotic cocktail was effective at depleting commensal flora, fresh fecal samples were collected and monitored for bacterial load. Subsequently, mice were evaluated for markers of inflammation and corneal epithelial cell damage. In addition, the effect of microbiota depletion on pathogenic T cell activation was addressed with CD4+ T cell adoptive transfer studies into T cell-deficient nude recipient mice.

Results: Microbiota-depleted C57BL/6 mice exposed to DS displayed a significant increase (p≤0.001) in the average number of CD4+ T cells in the conjunctiva (10.3±1.4), with a trend towards increased counts in the lids (25.8±6.1) when compared to DS mice with normal flora (conjunctiva: 7.5±0.9; lid: 15.7±3.7). Elevated CD4+ T cell infiltration in microbiota-depleted mice was associated with a significant increase (p≤0.001) in corneal fluorescein staining on Day 9 of DS (8.0±2.1) relative to staining in DS mice with normal flora (5.8±2.1).

Conclusions: These data suggest that alteration of the host microbiota increases the severity of DS-induced Dry Eye disease in mice.

Commercial Relationships: Chris S. Schaumburg, Allergan Inc. (E); Katherine S. Held, Allergan Inc. (E); Annie M. Ratanapinta, Allergan Inc. (E); Madhuri Paul, Allergan Inc. (E); Larry A. Wheeler, Allergan Inc. (E); Margarita Calonge, Allergan Inc. (C); Jerry Y. Niederkorn, Allergan Inc. (C); Stephen C. Pflugfelder, Allergan Inc. (C); Michael E. Stern, Allergan Inc. (E)

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Purpose: To assess the state of conjunctival tolerance in a murine model of evaporative dry eye and to evaluate the effect of topical nuclear factor kappa B (NFkB) modulation on disease progression.

Methods: A previously reported murine model of evaporative dry eye (Niederkorn JY et al. J Immunol. 2006 Apr 1;176(7):3950-7) was used with 8-week-old Balb/c mice (3 animals per treatment group). Ovalbumin (OVA) was used as a model antigen to assess immunological tolerance. In brief, OVA was instilled daily starting on days 1 and 4 in both eyes of mice under escopolamine- and air draft-induced desiccating stress for 5 days (DS5). In other experiment, topical NFkB inhibitors or vehicle were instilled 3 times a day during DS5. OVA specific T cell responses, as well as tear production and histopathological markers of disease, were evaluated. Conjunctival tolerance to OVA is expressed relative to the systemic T cell response of non-tolerant immunized mice.

Result: In the evaporative dry eye model (3 independent experiments), conjunctival tolerance to OVA was observed when antigen was instilled from day 1 (53±3%, p<0.05) but not when started on day 4 (110±20%). Mice treated with topical NFkB inhibitors while under DS5 exhibited greater tear production after DS5 than vehicle-treated mice (n=3, p=0.02) and recovered pretreatment levels of tear secretion before their untreated cagemates. Remarkably, hyperosmolarity did not induce NFkB activation in vitro in an epithelial cell line, and consistently, it did not increase epithelial expression of major histocompatibility complex II in cocultures with T cells.

Conclusions: Conjunctival tolerance is affected in mice under desiccating stress but only after 3 days of exposure, suggesting that ocular surface damage must reach a threshold in order to affect the immune outcome. As topical NFkB inhibitors during DS5 favor recovery of tear production afterwards, this signaling pathway, and conjunctival tolerance indirectly, must be implicated in disease progression. However, hyperosmolarity does not activate this signaling pathway in vitro, indicating that a more complex cellular & molecular interplay is involved in this model. Altogether these results highlight the therapeutic potential of conjunctival tolerance modulation in ocular surface disease.

Commercial Relationships: Jeremias G. Galletti, None; Mauricio Guzmán, None; Florencia Sabbione, None; Maria Laura Gabelloni, None; Silvia Vanzulli, None; Pablo A. Chiaradía, None; Javier F. Casiraghi, None; Analia S. Trevani, None; Mirta N. Giordano, None
Support: Fundación Allende and Fundación Alberto J. Roemmers (Argentina)
Purpose: CC Chemokine receptor 7 (CCR7) has emerged as a critical mediator of antigen-presenting cell (APC) migration from the cornea to the draining lymph node (LN). In this study, we investigated the effect of topical CCR7 blockade on the induction and maintenance of dry eye disease (DED), including the effect on Th17 immunity.

Methods: DED was induced in 6 to 8 week old female C57BL/6 mice through exposure to a controlled environment chamber (CEC) and scopolamine injections. To determine the functional role of CCR7 in the induction of DED, mice (n=10) were treated topically with either anti-CCR7, isotype antibody or remained untreated. Corneal fluorescein staining (CFS) was performed and mice were sacrificed on day 9 for flow cytometric quantification of IL-17 secreting CD4+ (Th17 cells) within the draining LN and real-time PCR analyses of IL-17, MMP-3, IL-1p and TNF-α expression at the ocular surface. In order to evaluate the effect of topical CCR7 blockade on the induction and maintenance of DED, mice (n=10) were re-exposed to the CEC after an initial 12 days of exposure to the CEC and a subsequent 10 days of being housed in room air. On re-exposure, the CEC mice were similarly treated topically with either anti-CCR7, isotype antibody or remained untreated and CFS was performed.

Results: Decreased Th17 frequencies in the draining LN and reduced conjunctival expression of IL-17 were observed in mice treated with topical anti-CCR7 compared to isotype treated mice. Ocular surface expression of MMP-3 and DED associated pro-inflammatory cytokines IL-1β and TNF-α were significantly decreased in the anti-CCR7 treated group (p < 0.05). Furthermore, significantly lower clinical fluorescein scores (CFS) were observed in anti-CCR7 treated mice compared to isotype treated mice (p < 0.001). Finally, topical CCR7 blockade was effective in ameliorating chronic DED (p<0.001).

Conclusions: Our findings show that CCR7 blockade is highly effective in inhibiting the immunopathogenesis of DED. Furthermore, these results suggest that CCR7 mediated trafficking of APCs drives the induction and maintenance of Th17 immunity in DED.

Commercial Relationships: Shilpa Kodati1, 2, Yihe Chen1, Thomas H. Dohlman1, Sunil K. Chauhan1, Daniel R. Saban1, Reza Dana1, Schepens Eye Research Institute and Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA; 2Ophthalmology, UPMC Eye Center, Pittsburgh, PA; 3Ophthalmology, Duke University, Durham, NC.
eye symptomatology by reducing inflammation. The T-cell mitogen Concanavalin A (ConA) has been previously utilized in a rabbit model of dry eye to study the potential of anti-inflammatory drugs (Nagelhout 2005). In this study, we optimized this ConA model in mice to create a modifiable inflammatory dry eye model.

**Methods:** Eyes of 10-12 week old female C57 mice were evaluated at baseline by corneal fluorescein staining and tear measurement with phenol red threads (ZoneQuick®). Animals were randomized based on their staining and tear volumes so that each group had a normalized baseline response. Mice were injected in the lacrimal gland with 100, 50, or 10µg/10µL of ConA or 10µg/10µL of phosphate buffered saline (PBS). An additional group of mice were treated with subcutaneous scopolamine and served as a positive control. For all studies, animals were placed in Ora’s controlled adverse environment (CAE) and were followed for 24 to 72 hours post-injection for corneal staining and tear measurement.

**Results:** ConA-injected mice showed a significant (p < 0.05), dose-dependent increase in corneal staining 24 hours post-injection when compared to the PBS-injected mice. Staining scores were 11.2, 7.0, 3.3 and 2.9, respectively, for the 100µg-, 50µg-, 10µg-, and 0µg-ConA injected animals. Staining declined from 24 to 48 hours, and returned to baseline for all groups by 72 hours. All ConA-injected mice showed a significant (p < 0.05) decrease in tear volume at 24 hours post-injection as compared to PBS-injected mice; these decreases returned to control values by 72 hours post-injection, and were not dose-dependent.

**Conclusions:** Our study shows that ConA injection can evoke an inflammatory reaction in the lacrimal gland leading to increases corneal staining and reduction in tear production. Interestingly, the effect on corneal staining is independent of the extent of tear gland suppression, and more dependent on the degree of inflammation. Our study establishes that ConA-induced inflammation in mice, in conjunction with CAE, can produce an acute form of dry eye disease that is modifiable and applicable for studying novel dry eye drugs.

**Commercial Relationships:** Laura Belen, Ora, Inc (E); Kortni Violette, Ora, Inc (E); George W. Ousler, Ora, Inc (E); Andy Whitlock, Ora, Inc (E)

Program Number: 3665 Poster Board Number: A0179
Presentation Time: 3:45 PM–5:30 PM

**Increased Substance P Expression in the Ocular Surface in Murine Dry Eye Disease**  
Sang-Mok Lee1, 2, Zahra Sadrai1, Hyun Soo Lee3, William Stevenson4, Yihe Chen1, Jing Hua1, Kishore Reddy Katikireddy1, Thomas H. Dohlman1, Sunil K. Chauhan1, Reza Dana1

**Methods:** DED was induced in 6-week-old female C57BL/6 mice by bilateral extra-orbital lacrimal gland excision followed by their placement in a controlled environmental chamber. Corneal fluorescent staining (CFS) was evaluated at days -1, 3, 6, and 13, and corneas and conjunctivae were harvested at days 4, 7, and 14 to analyze protein and mRNA expression levels of substance P. Substance P protein expression levels were measured with a competitive enzyme immunooassay kit (R&D Systems, Minneapolis, MN) and then adjusted to the total protein amount. Expression of substance P mRNA was analyzed by real-time PCR. One way ANOVA test was used for comparison of the expression levels and Pearson correlation was used for correlation analysis.

**Results:** Substance P protein expression levels were increased in both the cornea and conjunctiva on days 4 and 7 compared to the sham operated control group (Cornea: 1.9 fold on day 4, 2.0 fold on day 7, P<0.05; Conjunctiva: 2.8 fold on day 4, 2.2 fold on day 7, P<0.05). By day 14, levels of substance P decreased to baseline levels. We observed similar kinetics for the mRNA expression of substance P. Increased protein expression of substance P in the cornea correlated with increased CFS scores until day 7 (r = 0.900, P<0.001).

**Conclusions:** Protein and mRNA expression levels of substance P are increased in the early induction phase of DED and correlate with increased CFS scores.

**Commercial Relationships:** Sang-Mok Lee, None; Zahra Sadrai, None; Hyun Soo Lee, None; William Stevenson, None; Yihe Chen, None; Jing Hua, None; Kishore Reddy Katikireddy, None; Thomas H. Dohlman, None; Sunil K. Chauhan, None; Reza Dana, None

Support: NIH Grant R01 EY20889

Program Number: 3665 Poster Board Number: A0179
Presentation Time: 3:45 PM–5:30 PM

**REDUCED NUMBER AND ALTERED FUNCTIONAL ACTIVITY OF MOUSE CORNEAL COLD SENSORY NERVE FIBERS WITH AGE DEVELOP IN PARALLEL WITH DECREASED BASAL TEARING**

Omar González González1,2, Ignacio Alcalde1,2, Almudena Ibígo-Portugués1,2, Juana Gallar1, Jesús Merayo-Lloves1,2, Carlos Belmonte1,2, Fundacion Investigacion Oftalmologica, Oviedo, Spain; 2Universidad de Oviedo, Oviedo, Spain; 3Instituto de Neurociencias Universidad Miguel Hernandez-CSIC, San Juan de Alicante, Spain

**Purpose:** To determine in mice the effect of ageing on the number, morphology and electrophysiological properties of corneal cold sensory nerve axons and their influence on basal tearing rate.

**Methods:** TRPM8-EYFP mice of different ages (3, 6, 9, 12, 18 and 24th months) were studied. Basal tearing was measured in anesthetized animals, using phenol red threads. Corneal nerves expressing EYFP protein and neuronal class III beta-tubulin were identified in whole mount corneas using immunocytochemical techniques. Density of subbasal nerve branches and epithelial terminals was measured in the peripheral and central regions of the cornea. Trigeminal ganglion (TG) corneal neurons, labelled with fast blue applied onto the cornea in anesthetized mice, were identified in TG sections using immunofluorescence and counted. Extracellular electrical activity of single sensory nerve endings of the corneal surface was recorded in excised and superfused eyes.

**Results:** In 3-months mice, TRPM8+ subbasal nerve fibers represent 22.4% of the total number of sub-basal nerve branches; most of them were beaded axons finally ramifying in the uppermost epithelium as a cluster of beaded nerve terminals. Less abundant, longer and narrower fibers lacking beads and ending as a single or double bulbous terminal branch were also found. The total number of TRPM8+ subbasal nerve filaments and epithelial terminals decreased non-linearly with age. 3-months mice had 37% of low-threshold (>30.5 °C) cold sensitive fibers showing high background activity and vigorous firing responses to cooling. They were reduced to 26% in 24-months-old mice. Contrarily, cold sensitive fibers with a high cooling threshold (<30.5 °C), very low frequency background activity and weak responses to cooling was 17% in 3-months-old mice and 40% in 24-months-old mice. Basal tearing values also decreased with age (3months= 1.9 ± 0.2 mm; 24months= 1.3 ± 0.2 mm) varying in

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parallel with the number of TRPM8 subbasal branches and epithelial terminals.  

**Conclusions:** Reduction in the number and branching pattern of corneal low-threshold cold sensitive fibers with age seems to be associated with a lowering of the tearing rate, thus supporting the hypothesis that sensory input from ocular surface cold thermoreceptors contributes to the maintenance of basal tearing.

**Commercial Relationships:** Omar González González, None; Ignacio Alcalde, None; Almudena Íñigo-Portugués, None; Juana Gallar, None; Jesus Merayo-Lloves, None; Carlos Belmonte, None

**Support:** Fundación María Cristina Masaveu-Peterson, Fundación Ramón Areces, Caja Rural de Asturias and Bfu/2008-04425 and SAF2011-22500.

**Program Number:** 3666 Poster Board Number: A0180  
**Presentation Time:** 3:45 PM–5:30 PM  
**Autoimmune regulator (Aire) gene deficient mice, a model of autoimmune-mediated, aqueous-deficient dry eye, exhibit functional alteration of a recently demonstrated, special type of corneal nerves involved in basal tearing, referred to as the dry-sensitive corneal afferents**  

Harumitsu Hirata1, Thirugnana Vijmasi2, Mark I. Rosenblatt3, Nancy A. McNamara4, 1Ophthalmology, Weill Cornell Medical College, New York, NY; 2F1 Proctor Foundation, University of California at San Francisco, San Francisco, CA.

**Purpose:** Many of the symptoms and signs of aqueous deficient dry eye, such as reduced tearing and ocular neuropathy, are manifested in, Aire-knockout (KO) mice. To investigate the contribution of dry-sensitive neurons to the pathogenesis of dry eye disease (DED), we examined the response characteristics of these corneal neurons to a variety of ocular stimuli important in basal tear production.

**Methods:** In ketamine/xylazine (supplemented with isoflurane)-anesthetized mice, single extracellular recordings were made from trigeminal ganglion cells innervating the cornea while the cornea was stimulated with ocular dryness, menthol solutions and temperature changes. The corneal neurons excited by drying of the cornea (dry-sensitive corneal afferents) were studied in wild type (WT) and Aire-KO mice.

**Results:** In WT mice, the dry-sensitive corneal afferents were found within 30 min – 2 hrs after the start of the electrophysiological recordings. In line with our previous studies of corneal neurons in rats, the dry-sensitive neurons in WT mice showed vigorous discharges to drying of the cornea (and quieted by wetting), menthol and cooling stimuli applied to the ocular surface. By contrast, thus far, we have not been able to find the dry-sensitive corneal afferents in Aire-KO mice even after 3 hrs of recording and after demonstrating the presence of mechanoreceptors innervating the upper eyelids which lie in close proximity to dry-sensitive neurons. We are currently treating the Aire-KO mice with dexamethasone to attempt to reverse the effect of the deleterious ocular inflammation. We predict the presence of dry-sensitive neurons in the corneas of KO mice following anti-inflammatory therapy.

**Conclusions:** Our results demonstrate that the corneas of Aire-KO mice may be devoid of afferents whose activation is critical in maintaining ocular homeostasis by basal tear production, suggesting functional denervation of corneal nerves. We hypothesize that the neuronal loss in these mice with autoimmune disease is the direct result of chronic inflammation mediated by CD4+ T cells. Unraveling the mechanisms of how autoimmunity is associated with inflammation and corneal denervation, may provide a new avenue of treatments for DED.

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**Commercial Relationships:** Harumitsu Hirata, None; Thirugnana Vijmasi, None; Mark I. Rosenblatt, None; Nancy A. McNamara, None

**Support:** NIH Grants: EY020667; EY023555; RO1EY016203; EOY2162; and Research to Prevent Blindness Unrestricted Grant to Ophthalmology Department of Cornell Medical College

**Program Number:** 3667 Poster Board Number: A0181  
**Presentation Time:** 3:45 PM–5:30 PM  
**Secretary Group Two A Phospholipase (sPLA2-IIa) Expression and Function in Ocular Surface of Dry Eye Disease (DED) Mice Yi Wei, Pengcheng Li, Zhen Du, Disi Chen, Seth P. Epstein, Penny A. Asbell. Ophthalmology, Icahn School of Medicine at Mount Sinai, New York, NY.

**Purpose:** sPLA2-IIa has been demonstrated as a modulator of ocular surface (OS) inflammation and a biomarker of DED. Previous results on DED mice indicate that sPLA2-IIa is normally expressed in the fornix fold by the goblet (GC) and conjunctival (CNJ) epithelial cells but greatly induced by desiccation. However, the distribution of GCs is beyond the fornix of CNJ and does not fully overlap with that of sPLA2-IIa, and its role on cornea (CN) epithelia has not been clarified yet. The purpose of this study is to characterize the sPLA2-IIa-+ cells in OS of DED mice.

**Methods:** DED was induced on BALB/C mice as previously reported. Tear volumes were measured and ocular surface fluorescence staining were performed by masked ophthalmologists on Days 0, 4 and 7. Immunofluorescence assays (IFAs) were conducted on whole-mounted fresh eye samples or on frozen sections of inside-out or right-side-out eye samples.

**Results:** Single or double staining with primary antibodies against sPLA2-IIa and/or biomarkers of goblet cells (Muc5AC), epithelial cells (CK-7) and infiltrated inflammatory cells (CD11b, CD45 or Gr-1) were performed. (1) sPLA2-IIa+ signals are mainly found lining the surface of or within CNJ epithelial layer at fornix fold as previously reported; but heavy sPLA2-IIa+ staining can also be found on wounded CN epithelial spots, some even penetrating Stroma to reach to endothelial layer— this has never been reported before; (2) most heavy staining signals of sPLA2-IIa co-localize with that of Muc5AC lining the CNJ epithelia at the fornix fold, some do not overlap with each other; (3) sPLA2-IIa+ signals weakly overlap with CK7+ signals; (4) sPLA2-IIa+ signals barely overlap with the CD11b+, CD45+ or Gr-1+ signals, these infiltrated inflamed cells are restrained by CNJ epithelia barrier.

**Conclusions:** The heavy staining sPLA2-IIa+ cells separate from the infiltrated inflammatory responding cells may indicate a novel mechanism of sPLA2-IIa enzymes that pre-exist in the tear-film and OS to modulate immunity both in normal conditions and under stress. The finding of sPLA2-IIa accumulating at wounded corneal epithelial-defects may imply a possible new role of sPLA2-IIa in corneal wound healing and remodeling.

**Commercial Relationships:** Yi Wei, None; Pengcheng Li, None; Zhen Du, None; Disi Chen, None; Seth P. Epstein, None; Penny A. Asbell, None

**Support:** This study was supported in part by the Martin and Toni Sosnoff Foundation and Research to Prevent Blindness.
Purpose: Methylglyoxal (MGO) derived from New Zealand’s native Manuka honey (MH), demonstrates antibacterial properties that may be beneficial in the treatment of blepharitis. MH complexed with α-cyclodextrin increases bioavailability of the active ingredient (Manuka Honey CycloPower™ (MHCP), Manuka Health, NZ). With safety previously confirmed in vitro, this project sought to undertake in vivo safety testing of a novel formulation designed for topical eyelid application, containing MHCP in a microemulsion (ME) base.

Methods: Six male NZ white rabbits were administered 20μl of 10% MHCP (100MGO) in ME to the right eye, (diluted 1:10 in PBS as estimate of potential tear film contamination), and 20μl of saline (0.9% NaCl) to the left eye, instilled directly into the conjunctival sac daily, on 5 consecutive days. Tear film and ocular surface characteristics were compared before and after instillation, daily. Lipid layer grade, tear evaporation rate, osmolarity and production were assessed as well as ocular surface fluorescein staining, conjunctival hyperemia and corneal clarity. Subsequently, following washout, 20μl of undiluted formulation was instilled into one eye of each rabbit to confirm safety, and the ocular surface evaluated on the same day, after 0.5, 5 and 10 min.

Results: No statistically or clinically significant changes in lipid grade, tear production, evaporation rate, fluorescein staining or hyperemia were observed in either eye, across the 5 days (Repeated measures ANOVA/Friedman, p>0.05). Mean osmolarity decreased following instillation of formulation and control drops, but excepting Day 1 for MHCP (p=0.027), differences were not statistically significant (p>0.05). Bulbar hyperemia and lowered tear osmolarity induced by the undiluted formulation returned to baseline levels within 10 min.

Conclusions: Instilled at a concentration as high as 10% on 5 consecutive days, the MHCP formulation showed no significant adverse immediate or cumulative effects. Exposure to the tear film and ocular surface is unlikely to reach such high levels clinically when the preparation is applied topically to the eyelids. Instillation of the undiluted preparation, simulating accidental in-eye application, was transient and not associated with significant discomfort. The novel MHCP formulation appears safe and thus continues to show potential for development as a preparation for managing blepharitis.

Commercial Relationships: Jennifer P. Craig, Manuka Health NZ Ltd (F); Iva D. Rupenthal, Manuka Health NZ Ltd (F); Ali Seyfoddin, Manuka Health NZ Ltd (F); Amy Chen, Manuka Health NZ Ltd (F); Grant Watters, Manuka Health NZ Ltd (F)

Support: Unrestricted research grant, Manuka Health NZ Ltd

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**Eye Preparation for Blepharitis**

**Presentation Time:** 3:45 PM–5:30 PM

**In vivo rabbit tolerability and safety of a Manuka honey-based eye preparation for blepharitis**

Jennifer P. Craig1, Iva D. Rupenthal1, Ali Seyfoddin2, Amy Chen1, 3, Grant Watters1, 4.

1Ophthalmology, University of Auckland, Auckland, New Zealand; 2School of Pharmacy, University of Auckland, Auckland, New Zealand; 3Optometry and Vision Science, University of Auckland, Auckland, New Zealand.

**Purpose:** Methylglyoxal (MGO) derived from New Zealand’s native Manuka honey (MH), demonstrates antibacterial properties that may be beneficial in the treatment of blepharitis. MH complexed with α-cyclodextrin increases bioavailability of the active ingredient (Manuka Honey CycloPower™ (MHCP), Manuka Health, NZ). With safety previously confirmed in vitro, this project sought to undertake in vivo safety testing of a novel formulation designed for topical eyelid application, containing MHCP in a microemulsion (ME) base.

**Methods:** Six male NZ white rabbits were administered 20μl of 10% MHCP (100MGO) in ME to the right eye, (diluted 1:10 in PBS as estimate of potential tear film contamination), and 20μl of saline (0.9% NaCl) to the left eye, instilled directly into the conjunctival sac daily, on 5 consecutive days. Tear film and ocular surface characteristics were compared before and after instillation, daily. Lipid layer grade, tear evaporation rate, osmolarity and production were assessed as well as ocular surface fluorescein staining, conjunctival hyperemia and corneal clarity. Subsequently, following washout, 20μl of undiluted formulation was instilled into one eye of each rabbit to confirm safety, and the ocular surface evaluated on the same day, after 0.5, 5 and 10 min.

**Results:** No statistically or clinically significant changes in lipid grade, tear production, evaporation rate, fluorescein staining or hyperemia were observed in either eye, across the 5 days (Repeated measures ANOVA/Friedman, p>0.05). Mean osmolarity decreased following instillation of formulation and control drops, but excepting Day 1 for MHCP (p=0.027), differences were not statistically significant (p>0.05). Bulbar hyperemia and lowered tear osmolarity induced by the undiluted formulation returned to baseline levels within 10 min.

**Conclusions:** Instilled at a concentration as high as 10% on 5 consecutive days, the MHCP formulation showed no significant adverse immediate or cumulative effects. Exposure to the tear film and ocular surface is unlikely to reach such high levels clinically when the preparation is applied topically to the eyelids. Instillation of the undiluted preparation, simulating accidental in-eye application, was transient and not associated with significant discomfort. The novel MHCP formulation appears safe and thus continues to show potential for development as a preparation for managing blepharitis.

**Commercial Relationships:** Jennifer P. Craig, Manuka Health NZ Ltd (F); Iva D. Rupenthal, Manuka Health NZ Ltd (F); Ali Seyfoddin, Manuka Health NZ Ltd (F); Amy Chen, Manuka Health NZ Ltd (F); Grant Watters, Manuka Health NZ Ltd (F)

**Support:** Unrestricted research grant, Manuka Health NZ Ltd

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**Effect of Three Tear Supplements on Signs, Symptoms and Pro-inflammatory Markers in Subjects with Dry Eye Disease**

Eilidh Martin1, 4, Suzanne Hagan1, 4, Katherine Oliver1, 4, Ian Pearce1, 4, Alan Tomlinson1.

1Vision Sciences, Glasgow Caledonian University, Glasgow, United Kingdom; 2Biological Sciences, Glasgow Caledonian University, Glasgow, United Kingdom.

**Purpose:** A preliminary study to investigate the effect of three commercial tear supplements (Optive Plus - Allergan, Refresh Contacts - Allergan and Systane Balance - Alcon) when used in dry eye patients for one week. The signs and symptoms of dry eye disease, along with inflammatory status pre- and post-treatment, were compared for each tear supplement.

**Methods:** Eighty dry eye subjects were recruited (Schirmer ≤10mm in 5 minutes), comprising 14 females and 4 males, with a mean age of 52.8 years (±15.8). In a crossover design subjects received each treatment for 1 week (4 times a day), followed by 1 week washout, where subjects received no treatment. At each visit, ocular surface disease index questionnaire (OSDI), tear evaporation rate, tear stability, tear film interferometry, tear osmolarity and tear samples were taken. All of these measures, except tear film interferometry, were carried out in an environmental chamber at 22°C and 20% relative humidity.

**Results:** A reduction was observed in the concentrations of all 7 cytokines measured (IL-1β, IL-2, IL-6, IL-8, IL-17, IFN-γ and TNF-α) post treatment, with each of the products tested. The greatest percentage reduction was seen in IL-17 for both Refresh Contacts and Systane Balance, with a median of -43.1% and -83.8%, respectively. TNF-α showed the greatest percentage reduction (-42.2%), for Optive Plus, however, none of these reductions were statistically significant. Interestingly, the changes to all 7 cytokines showed significant correlations with each other, i.e. as one cytokine’s concentration reduced, there was a corresponding change in all of the others. No statistically significant change was observed pre- and post- treatment for any of the clinical measurements used, except for a significant improvement in OSDI with Refresh Contacts (p=0.012).

**Conclusions:** A consistent trend was observed for a reduction in the levels of all 7 cytokines measured following 1 week of treatment. Although the cytokines were reduced, this was not reflected in a reduction in clinical signs and symptoms. This could indicate a time-lag between inflammatory status and clinical improvement, or a lack of sensitivity on the part of the clinical tests used. Further work with a larger patient population, longer duration of treatment, and more specificity as to dry eye etiology may be useful to extend and confirm the present results.

**Commercial Relationships:** Eilidh Martin, Allergan Inc (R); Suzanne Hagan, Allergan Inc (F), FIGHT for Sight (F); Katherine Oliver, Allergan Inc (F); Ian Pearce, None, Alan Tomlinson, Allergan Inc (F)

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**Effect of Lachrymal Substitutes on Tear Film Thickness in Patients with Moderate Dry Eye Syndrome**

Doreen Schmidt1, 2, Katarzyna J. Napora1, 2, René M. Werkmeister1, Peter Rosner1, Gerhard Garhofer1, Leopold Schmetterer1, 2, Doreen Schmidt1, 2, Katarzyna J. Napora1, 2, René M. Werkmeister1, Peter Rosner1, Gerhard Garhofer1, Leopold Schmetterer1, 2.

1Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria; 2Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria.

**Purpose:** The aim of the present study was to test the effect of a single administration of three different lachrymal substitutes on tear film thickness in patients with moderate dry eye syndrome (DES).

**Methods:** The study was carried out in a randomized, double-masked, active-controlled design. 60 patients with moderate DES were included and randomized to receive either eye drops containing unpreserved trehalose combined with sodium hyaluronate, eye drops containing unpreserved sodium hyaluronate or sodium chloride eye drops. Tear film thickness (TFT) was measured using an ultrahigh-resolution optical coherence tomography (OCT) system. Measurements were performed pre-instillation and 10 minutes, 20 minutes, 40 minutes, 60 minutes, 120 minutes and 240 minutes after
Autologous Serum for Dry Eye Treatment

Purpose: To assess a clinical difference in the management of dry eye between autologous serum (AS) and plasma rich in growth factors (PRGF).

Methods: We made a comparison between the clinical efficacy of autologous PRGF and autologous serum in patients diagnosed with dry eye, who did not respond previously to other standard treatments, in terms of symptoms (OSDI questionnaire). An objective evaluation by slit lamp (tear film rupture, tear meniscus height) and Schirmer Test was made. Following the recommendations of the Helsinki Declaration and approval by the Hospital de la Luz Ethical Committee, we obtained the informed consent of these patients before commencing the study.

Results: At the moment we have 7 patients treated with AS and artificial tears (Group A), 3 patients with PRGF and artificial tears (Group B), and 10 with artificial tears only (Group C). In Group A, after 1 month follow-up, we found an average decline of 1 level in dry eye severity, no changes in TFR and meniscus height, Schirmer test in males was 1 mm more in both eyes, in females was 2.64 mm for right eye and 1.5 mm for left eye, in the OSDI score males had a reduction of 19.5 points and females of 20.39 points.

Conclusions: All groups had significant clinical improvement, even though we observed an inconsistency between the clinical data and the OSDI score. The group B improvement was remarkable, compared with other groups, but we need a larger sample size for a statistical analysis.

Commercial Relationships: Edna Lucia Valdez Payan, None; Oscar Fernandez, None; Regina Velasco, None; Oscar Baca, None; Alejandro Babayan, None; Cristina Pacheco Del Valle, None; Elisa D. Alegria, None; Atzin Robles-Contreras, None
Clinical Trial: 0165

Program Number: 3672 Poster Board Number: A0186
Presentation Time: 3:45 PM–5:30 PM
Reduced Rescue Artificial Tear Use in Subjects Using a Topical Interleukin-1 (IL-1) Receptor-1 (R1) Blocker for Ocular Treatment of Dry Eye Disease (DED)

Michael H. Goldstein1,2, Gregory Zarbis-Papastoitis2, Kathryn Golden1, Cameron Wheeler1, Joseph Kovalchin1, Jennifer Agaghiian1, Karen Tubridy1, Abbie Celniker2, Eric S. Furfine2, Ophthalmology, New England Eye Center, Boston, MA; 1Eleven Biotherapeutics, Cambridge, MA.

Purpose: In therapeutic studies in dry eye disease (DED), the placebo response is well documented with a magnitude of effect of 20-35% on signs and symptoms of DED compared with baseline. This response is believed to be principally a result of two components: the placebo response typically seen in therapeutic drug trials and the wetting or lubricating effect of the topically applied vehicle. A third component, however, may play an important role in the vehicle response: the use of rescue artificial tears.

Methods: EBI-005 is a novel, potent IL-1R1 inhibitor that was rationally designed to treat ocular surface disorders, such as DED. In a double-masked, placebo-controlled study, 74 subjects with moderate to severe DED were randomized to receive vehicle control or EBI-005 (5 or 20 mg/mL). Subjects received the study medication 3x/day for 6 weeks and were allowed to use rescue artificial tears provided by the sponsor (Refresh plus®, Allergan Pharmaceuticals, Irvine, CA). Subjects recorded the number of vials of artificial tear used in a diary and were not allowed to use rescue tears within two hours of dosing of the study medication.

Results: Mean rescue artificial tear use over the six week study period was 11.1 vials for subjects receiving EBI-005 and 31 vials for subjects receiving vehicle control (p value=.005). Median rescue artificial tear use over the six week period was 1 vial for subjects receiving EBI-005 and 10.5 vials for subjects receiving vehicle control. There was a lower percentage of users of large amounts of rescue artificial tears (defined as user of more than 50 vials during the 6 week study period) among subjects receiving EBI-005 (5% or 2 of 39) compared with vehicle control (35% or 9 of 26) (p value=.005). Of the 10 heaviest artificial tear users, eight (80%) were subjects receiving vehicle.

Conclusions: Subjects treated with EBI-005 used fewer rescue artificial tears than vehicle control treated subjects over the six week study period. Increased use of rescue artificial tears may play a key role in the magnitude of the vehicle response seen in many DED studies. Although not a currently acceptable regulatory endpoint in the United States, reduction of rescue artificial tear use by a study medication may have an important pharmacoeconomic impact that warrants further study.

Commercial Relationships: Michael H. Goldstein, Eleven Biotherapeutics (C); Gregory Zarbis-Papastoitis, Eleven Biotherapeutics (E); Kathryn Golden, Eleven Biotherapeutics

Program Number: 3672 Poster Board Number: A0186
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Commercial Relationships: Michael H. Goldstein, Eleven Biotherapeutics (C); Gregory Zarbis-Papastoitis, Eleven Biotherapeutics (E); Kathryn Golden, Eleven Biotherapeutics

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Presentation Time: 3:45 PM–5:30 PM
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Commercial Relationships: Michael H. Goldstein, Eleven Biotherapeutics (C); Gregory Zarbis-Papastoitis, Eleven Biotherapeutics (E); Kathryn Golden, Eleven Biotherapeutics

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**Clinical Trial:** NCT01748578

**Program Number:** 3673 Poster Board Number: A0187

**Presentation Time:** 3:45 PM–5:30 PM

**SYL1001, a new treatment based on RNAi for the treatment of ocular pain**

Veronica Ruz1, Victoria Gonzalez1, Carmen Martinez-Garcia2, Covadonga Pañeda1, Ana Isabel Jiménez1. 1Sylentis, Madrid, Spain; 2Universidad de Valladolid, Valladolid, Spain.

**Purpose:** SYL1001 is a small interfering RNA targeting transient receptor potential cation channel subfamily V member 1 (TRPV1). The compound, administered in eye drops, is undergoing clinical development for the treatment of ocular pain. SYL1001 decreases TRPV1 in animal models and reduces pain related behavior in an animal model of capsicain induced eye pain.

**Methods:** Biodistribution studies for SYL1001 and its active metabolite were performed in New Zealand rabbits. Five minutes after administration the ocular structures were harvested: cornea, conjunctiva and lachrymal gland. The trigeminal/semilunar ganglion was collected to assess if the compound was able to reach the neurons innervating the cornea. Lung, liver, kidneys and plasma were collected to assess systemic exposure. Phase 1A in healthy subjects was completed in 2011 and a phase 1/2 clinical trial is currently ongoing. SYL1001_1 (NCT01438281) was an open-label, controlled, single-centre, phase 1 study that assessed safety of SYL1001 in 30 healthy subjects. SYL1001_II (NCT1776658) is a double-masked, controlled, multi-center, phase 1/2 study that studies safety and effect of SYL1001 in 60 patients with ocular pain.

**Results:** Results of the biodistribution studies show that SYL1001 and its active metabolite are present in cornea, conjunctiva, lachrymal gland and trigeminal ganglion; structures were SYL1001 is believed to exert its action. SYL1001 was detected in plasma and the active metabolite of SYL1001 was not present in plasma or systemic organs. TRPV1 protein is expressed in different eye cells from rabbit and human eyes, particularly active in Ca2+ exchange as well as in cells with significant water transport activity. Clinical trials showed that local tolerance was excellent. No serious adverse or modifications of the ocular surface or iris were detected. The analytical results at final examination did not show differences from those observed during selection. Pharmacokinetic results indicated that no levels of siRNAs were detected above in any of the subjects.

**Conclusions:** Preclinical and clinical development of SYL1001 indicates that the compound is very well tolerated both locally and systemically. SYL1001 was not present in any of the blood samples collected in humans after either a single or repeated dosing schedule; the absence of the compound from systemic circulation indicates that the action of SYL1001 is most likely localized.

**Commercial Relationships:** Veronica Ruz, Sylentis (E), Sylentis (I); Victoria Gonzalez, Sylentis (E), Sylentis (I); Carmen Martinez-Garcia, None; Covadonga Pañeda, Sylentis (E), Sylentis (I); Ana Isabel Jiménez, Sylentis (E), Sylentis (I)

**Support:** CDTI- CENIT program CeyeC

**Clinical Trial:** NCT01776658

**Program Number:** 3675 Poster Board Number: A0189

**Presentation Time:** 3:45 PM–5:30 PM

**GML inhibits lipase production by ocular isolates**

Neeta Khandekar1, Judith Flanagan1,2, Keizo Watanabe1,2, Rani S. Bandara1, Amali Ariyavardana1, Brien A. Holden1,2, Eric B. Papas1,2, Hua Zhu1,2, 1Brien Holden Vision Institute, Sydney, NSW, Australia; 2School of Optometry and Vision Sciences, UNSW, Sydney, NSW, Australia; 3Department of Ophthalmology, Kinki University Faculty of Medicine, Osaka, Japan.

**Purpose:** Evaporative dry eye is usually linked to an unstable tear film. Alterations in the meibomian lipids can cause evaporation of the tear film and exacerbate dry eye conditions. Lipase produced...
by commensal ocular bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis* might play a role in dry eye by degrading meibomian lipids. Glycerol monolaurate (GML) is known to inhibit lipase production by Gram positive bacteria including *S. aureus* on skin and mucosal surfaces. We studied the effect of GML in inhibiting lipase production by ocular isolates in addition to its effect on bacterial cell growth.

**Methods:** Concentrations of GML from 5 µg/mL – 25 µg/mL were prepared in Tryptic Soy Broth (TSB). Test strains *S. aureus* 020 & 134, and *S. epidermidis* 001 & 024 were inoculated at the density of 10^6 in varying concentrations of GML for 24 h at 37°C with constant shaking. After 24 h, the bacterial suspensions were centrifuged and the supernatant was analyzed for presence of bacterial lipases using commercial Lipase assay kit. Bacterial cell growth was assessed by measuring OD at 660 nm. The test was conducted 3 times.

**Results:** GML inhibited lipase production by test strains in a dose-dependent manner, with 50% (±25%) inhibition at 10 µg/mL and 88% (±7%) at 17.5 µg/mL for *S. aureus* 020; and 25% (±21%) at 10 µg/mL and 63% (±9%) at 17.5 µg/mL for *S. aureus* 134. For *S. epidermidis* 001 the inhibition was 28% (±14%) at 7.5 µg/mL and 43% (±12%) at 12.5 µg/mL; whilst, for *S. epidermidis* 024, it was 52% (±7%) at 7.5 µg/mL and 64% (±22%) at 12.5 µg/mL. GML showed significant (p < 0.05) lipase inhibition above concentrations of 15 µg/mL in *S. aureus* 020 and *S. aureus* 134, without exhibiting antimicrobial activity for the tested concentrations. For *S. epidermidis* 001 and *S. epidermidis* 024, GML showed significant (p < 0.05) lipase inhibition above 10 µg/mL and 7.5 µg/mL, respectively.

**Conclusions:** GML inhibits lipase production by *S. aureus* and *S. epidermidis* at low concentrations without adversely affecting bacterial cell growth. Therefore, GML in low concentrations can be used to inhibit lipases produced by ocular isolates without proving detrimental to commensal bacteria.

**Commercial Relationships:** Neeta Khandekar, None; Judith Flanagan, None; Keizo Watanabe, None; Rani S. Bandara, None; Amali Ariyawardana, None; Brien A. Holden, None; Eric Papas, None; Hua Zhu, None

**Program Number:** 3676

**Poster Board Number:** A0190

**Presentation Time:** 3:45 PM–5:30 PM

**VETERINARY CLINICAL INVESTIGATIONS: USE OF HETEROLOGOUS MESENCHYMAL STEM CELLS IN DOGS WITH KERATOCONJUNCTIVITIS SICCA**

Mauro K. Bittencourt1, Michele A. Barros1, Karine Evangelho1, Jose Paulo C. Vascenconcellos1, Joao Flavio P. Martins2, Matheus D. Bittencourt1, Cristiano V. Wenceslau1, Bruna P. Morais3, Irina Kerkis4, 1Department of Ophthalmology, Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil; 2Universidade Federal de São Paulo, São Paulo, Brazil; 3Universidad Nacional de Rio Cuarto, Rio Cuarto, Argentina; 4Instituto Butantan, São Paulo, Brazil; 5Department of Ophthalmology, Hospital Beneficência Portuguesa, São Paulo, Brazil; 6Universidade de São Paulo (USP), São Paulo, Brazil.

**Purpose:** To evaluate the use of heterologous mesenchymal stem cells (MSC) derived from adipose tissue in dogs with keratoconjunctivitis sicca (KSC).

**Methods:** MSCs were obtained under good manufacturing practice conditions and fully characterized. KSC was defined by quantitative Schirmer tear test (STT) below 15mm/min. Eleven dogs of different gender, age and race were enrolled in present study. One eye of each dog was treated with 10^6 MSCs which were administrated in a total dose of 0.5ml of physiologic solution. Each dose was applied into two sites: 0.3 ml of MSC solution was administered directly to the main lacrimal gland and 0.2 ml to the third eyelid gland. The eyes were evaluated weekly during 8 weeks using STT, fluorescein test and slit-lamp biomicroscopy. The severity of eye score (SES) was evaluated according to conjunctival hyperemia, ocular discharge, corneal opacity or irregularity and neovascularization. Dogs, which did not show significant improvement five weeks after MSCs application, were submitted to a second application following the same protocol and scheme of evaluation.

**Results:** After 3 weeks the dogs showed increased STT values when compared at baseline levels and were statistically significant (p = 0.0023) This increase of STT values remained significant until the 5th week of MSM application and 55% of the dogs showed improvement in tear production with STT measurements above
15 mm/min. The remaining animals, which needed to receive two applications of MCSSs, reached their peak of tear production at the 7th week, demonstrating statistically significant results as well (p = 0.003). After 8 weeks the dogs showed STT values increased, when compared with STT at the beginning of treatment (p = 0.0228). The clinical improvements of corneal opacity (p = 0.006) and conjunctival secretion (p = 0.0376) in eyes were also observed. However, the data obtained regarding the degree of conjunctival hyperemia and corneal vascularization were not statistically significant.

**Conclusions:** MSCs used in present study suggested their safety, once none of the animals demonstrated any type of rejection, allergic reaction or tumor formation. These cells demonstrated a clear clinical benefit in the treatment of KSC, thus improving the function of the lacrimal glands and of several other parameters. This study provides a basis for future clinical studies in humans with KSC.

**Commercial Relationships:** Maura K. Bittencourt, None; Michele A. Barros, Regenera Medicina Veterinária Avançada (I); Karine Evangelho, None; Jose Paulo C. Vascconcelos, None; João Flávio P. Martins, Regenera Medicina Veterinária Avançada (I); Mathieu D. Bittencourt, None; Cristiane V. Wenceslau, None; Bruna P. Moraes, Regenera Medicina Veterinária Avançada (E); Irina Kerkis, None

**Program Number:** 3678 **Poster Board Number:** A0192  
**Presentation Time:** 3:45 PM–5:30 PM

**Oral administration of Maqui berry (Aristotelia chilensis) extract restores tear secretion capacity in rat blink-suppressed dry eye model by modulating lacrimal gland function**

Shigeru Nakamura1, Juynji Tanaka2, Ryuji Hisamura3, Imada Yoshihiro4, Hiroshi Shimoda5, Kazuo Tsutoba4. Ophthalmology, Keio University, Tokyo, Japan; 1Research and Development, Oryza Oil & Fat Chemical, Ichinomiya, Japan.

**Purpose:** Maqui berry [Aristotelia chilensis (Molina) Stuntz] is a plant of the Elaeocarpaceae family and cultivated in central and southern Chile. Maqui berry has a particularly high concentration of anthocyanins. Many studies conducted on the biological activities of maqui berry extract (MBE) have reported, such as antioxidant, cardioprotective, anti-diabetic and anti-inflammatory. In this study, we investigate the effect of MBE on lacrimal function in VDT-associated dry eye using rat model.

**Methods:** 8-weeks-old female Sprague-Dawley rats were used for this study. A series of treatments were performed under continuous exposure to low humidity airflow (25±5%, 2-4m/s). Rats were placed on a swing made of a plastic pipe for 7.5h/d, and for 16.6hours, they were placed in individual cages without swing treatment. This series of treatments was repeated for up to 10 days. During swing procedures, rats received orally either MBE (40mg/kg) or water (vehicle), administered once daily for 10days. Change in tear secretion and corneal surface was measured by cotton thread test and applying a fluorescein solution under a blue-free barrier filter. Lacrimal glands (LG) were subject to assessment of ROS production by using the fluorescent probe 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate acetyl ester.

**Results:** A significant decreases in tear secretion were observed in vehicle group compared to initial value (p<0.001). In the MBE group, there were little changes in tear secretion compared with initial value. Changes in tear secretion were significantly suppressed in the MBE compared with vehicle (p<0.001) at day11. A significant increases in corneal fluorescein score were observed in vehicle compared initial value (p<0.001). In the MBE, slight increases in corneal fluorescein score were observed, but the differences were not significant compared with initial value. Changes in corneal fluorescein score were significantly suppressed in MBE compared with vehicle (p<0.001) at day10. The ROS production from LG was significantly suppressed in the MBE group compared to vehicle group (55.1±5.4% vs. vehicle, p<0.05).

**Conclusions:** These results indicate that MBE restored dry eye symptoms by acting on the LG and may represent a very potent nutritional treatment for the prevention of dry eye.

**Commercial Relationships:** Shigeru Nakamura, None; Juynji Tanaka, None; Ryuji Hisamura, None; Imada Yoshihiro, None; Hiroshi Shimoda, None; Kazuo Tsutoba, None

**Support:** ORYZA OIL & FAT CHEMICAL

**Program Number:** 3679 **Poster Board Number:** A0193  
**Presentation Time:** 3:45 PM–5:30 PM

**Correlation between mRNA and protein expression profiles of HLA-DR in Conjunctival Impression Cytology using a new device for collecting epithelial cells**

Karima KESSAL1, 3, Luisa Riancho1, 3, Ghislaine Rabut5, Hong Liang2, Celine Boucher2, Stephane Melik-Parsadaniantz1, 4, Christophe Baudouin2, Françoise Baudouin2, 4, 1Institut de la vision, UPMC UMR S968, Paris, France; 2Ophthalmologie III, CHNO XV-XX, Paris, France; 3Centre Hospitalier National d’Ophthalmologie des Quinze-Vingts, INSERM-DHOS CIC 503, Paris, France; 4Faculté des Sciences Pharmaceutiques et Biologiques, Université Paris Descartes, Sorbonne, Paris, France; 5UPMC U968, INSERM, Paris, France

**Purpose:** Human Lecucocyte Antigen (HLA-DR) expression in conjunctival impression cytology (CIC) is recognized as a reliable biomarker to follow various inflammatory ocular surface (OS) disorders. Flow Cytometry (FC) is routinely used to investigate the inflammatory level of OS through a quantification of HLA-DR antigen expression. Our aim was, using the new cell collection device EyePRIM (EP), to compare two different approaches for HLA-DR evaluation: FC for the surface protein assessment and Real time Polymerase Chain Reaction (PCR) for the gene expression with messenger ribonucleic acid (mRNA) analysis.

**Methods:** CIC samples were obtained from thirty (n=30) patients with various OS diseases and ten (n=10) normal subjects; Schirmer test, Tear break-up time and OSDI score were also performed. Sample collection of CIC specimens was performed using EP device. It is carried out by applying the polyethesulfone filter adapted in the device onto the anaesthetized bulbar conjunctiva for a few seconds, after which it was removed. Cells were harvested from CIC samples and subjected to FC (FC500, Beckman Coulter) using monoclonal antibodies directed to HLA-DR, and real-time PCR (Applied Biosystems) was performed in parallel allowing to quantitatively analyzing the protein expression and mRNA transcripts of HLA-DR respectively.

**Results:** Enough RNA materials were collected using EP device with an average of 1μg/CIC. All patients included had high HLA-DR levels compared to healthy volunteers with induction in HLA-DR transcripts and protein expression. Positive correlation was found between mRNA and protein expression of HLA-DR (R square = 0.57 Pearson r = 0.75), and significant correlation (p<0.0001) was observed for the whole dataset.

**Conclusions:** This study showed an overall positive correlation between mRNA and protein expression assessed with FC. Moreover, the quantity of RNA material collected with the EYEPRIM device allows further gene expression investigation. This device appears useful for the impression cytology and molecular markers investigation. Finally, these results, since HLA-DR is a marker of OS inflammation, suggest that the relationship and correlation between protein and transcripts of other ocular diseases biomarkers need to be explored.

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Purpose: To develop a continuous blink tracking device and investigate diurnal blink as a clinical endpoint.

Methods: Blink data were collected using a wearable telemetric device to record electromyograms corresponding to activity of the orbicularis oculi muscle. Subjects with no history of ocular disease were tested for verification and validation of the diurnal blink device. Verification testing was performed to ensure that the device accurately recorded blink rate compared to manually recorded blink activity from video capture (N=7). Validation testing of the device was performed to examine the variability of hourly and daily repeated measurements (N=9). Finally, the effect of exposure to a 90 minute controlled adverse environment (CAEM™) challenge and an exaggerated 30 minute CAEM™ challenge on blink rate (N=6) was examined.

Results: In the verification study, the mean blink rate was 10.79 blink/minute (standard deviation, SD 3.48) with the device versus 10.61 (SD 4.01) with manual counting. In the validation study, mean daily blink rates and IBIs, calculated to test for daily variability, were not significantly different from day 1 to day 2 (p=0.164 and p=0.060, respectively). On the first day, the mean blink rate was 14.22/minute (SD 6.28), IBI was 4.73 seconds (SD 1.41), and on the second day, mean blink rate was 11.61/minute (SD 3.28), IBI was 5.61 (SD 1.76). IBI during the 90-minute CAEM™ challenge were not significantly different compared to mean hourly IBIs before or after challenge (p>0.19). However, during the exaggerated CAEM™ challenge, mean IBI (3.5, SD 0.871) were significantly decreased compared to pre-CAEM™ values (4.54, SD 0.955)(p=0.026).

Conclusions: These preliminary data support the verification and validation of the diurnal blink tracking device. Additionally, the device has demonstrated its ability to successfully measure changes in blink patterns due to alternations in environmental conditions. These studies demonstrate that continuous blink tracking utilizing this method has potential applications in future dry eye studies.

Commercial Relationships: John D. Rodriguez, Ora, Inc. (E); Endri Angjeli, Ora, Inc. (E); Colleen Heckley, Ora, Inc. (E); Keith J. Lane, Ora, Inc. (E); George W. Ousler, Ora, Inc. (E)

Program Number: 3682 Poster Board Number: A0196

Presentation Time: 3:45 PM–5:30 PM

Tear Cytokines in Non-Dry Eye and Dry Eye Participants After Exposure to a Low Humidity Environmental Exposure Chamber Lakshman N. Subbaraman1, David J. McCanna1, Holly I. Lorentz2, Fiona Soong1, Anne Marie Salapatek2, Lyndon W. Jones1,1 CCLR, School of Optometry, University of Waterloo, Waterloo, ON, Canada; 2Inflammax Research Inc, Mississauga, ON, Canada.

Purpose: To quantify tear cytokine levels in Non-Dry eye (NDE) and Dry Eye (DE) participants before and after exposure to a Low Humidity-Environmental Exposure Chamber (LH-EEC).

Methods: Basal tear samples were collected (pre-exposure) from 8 NDE and 8 DE participants prior to their entry into the LH-EEC. The participants remained in the LH-EEC with controlled temperature (23±3°C), relative humidity (10±3%) and air velocity (3–5ft/sec) and performed visual tasking for 180 minutes. Prior to their exit, basal tears were collected (post-exposure). Subsequently, participants remained in the clinic and tears were collected again after 60 minutes (recovery). The collected tear samples were analyzed using the human pro-inflammatory V-plex assay (IFN-γ, IL-10, IL-12 p70, IL-13, IL-1β, IL-2, IL-4, IL-6, IL-8 and TNF-α) using the MesoScale Discovery Platform.

Results: There was a significant difference between the NDE and DE participants for IL-2 (p=0.009), however, no significant differences were seen between the two groups for other cytokines. The mean and
standard deviation for each cytokine in the NDE and DE participants under 3 conditions are provided in the Table. Exposure to the EEC caused over 100% increase in the cytokine concentration in some participants. **Conclusions:** Induction and reduction in tear cytokines vary after exposure to a controlled LH-EEC in NDE and DE participants. The LH-EEC model can be used to understand the role of tear film biomarkers in the pathogenesis of dry eye.

**Table:** Pre-exposure (pg/ml) Post-exposure (pg/ml) Recovery (pg/ml)

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<tr>
<th></th>
<th>DE</th>
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<th>DE</th>
<th>NDE</th>
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<tr>
<td>IFN-γ</td>
<td>0.34±0.24</td>
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<td>0.41±0.81</td>
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<td>0.54±0.5</td>
<td>0.16±0.1</td>
<td>0.52±0.5</td>
<td>1.02±0.4</td>
</tr>
<tr>
<td>IL-2</td>
<td>1.11±0.83</td>
<td>0.46±0.57</td>
<td>1.52±0.91</td>
<td>0.43±0.27</td>
<td>1.24±0.12</td>
<td>0.31±0.32</td>
</tr>
<tr>
<td>IL-4</td>
<td>0.49±0.12</td>
<td>0.36±0.17</td>
<td>0.49±0.14</td>
<td>0.43±0.15</td>
<td>0.36±0.21</td>
<td>0.39±0.16</td>
</tr>
<tr>
<td>IL-6</td>
<td>4.5±0.3</td>
<td>7.5±0.38</td>
<td>5.4±0.57</td>
<td>5.49±0.58</td>
<td>4.5±0.31</td>
<td>10.0±0.65</td>
</tr>
<tr>
<td>IL-8</td>
<td>27.3±0.18</td>
<td>27.6±0.18</td>
<td>22.27</td>
<td>3.1±0.18</td>
<td>17.0±0.18</td>
<td>352.2±0.18</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.14±0.39</td>
<td>1.30±0.34</td>
<td>1.24±0.35</td>
<td>1.03±0.25</td>
<td>1.37±0.82</td>
<td>1.26±0.34</td>
</tr>
</tbody>
</table>

Tear cytokine levels in NDE and DE participants under three different conditions

**Commercial Relationships:** Lakshman N. Subbaraman, NSERC Engage (F); David J. McCanna, None; Holly I. Lorenz, Inflamx Research (E); Fiona Soong, Inflamx Research (E); Anne Marie Salapatek, Inflamx Research (E); Lyndon W. Jones, NSERC Engage (F)

**Support:** NSERC Engage, Canada

**Program Number:** 3683 Poster Board Number: A0197

**Presentation Time:** 3:45 PM–5:30 PM

**Correlations between non-stimulated tear levels and conjunctival gene expression of MAPK pathway-associated biomarkers in aqueous-deficient dry eye and control patients**

**Roderick J. Fullard, Nicole M. Gayette, My-Tho K. Tran, John L. Bradley.** Vision Sciences, Univ of Alabama Birmingham, Birmingham, AL.

**Purpose:** Chronic hyperosmolarity at the ocular surface increases tear levels of pro-inflammatory cytokines IL-6, IL-1β, IL-8, TNF-α, and G-CSF and activates MAPK signaling pathways. Concurrent changes in conjunctival MAPK-associated gene expression are not clearly defined. The current study compares tear levels of MAPK-associated biomarkers with concurrent conjunctival gene expression of related biomarkers in aqueous-deficient dry eye (ADDE) and control patients.

**Methods:** Patients were allocated to three groups by Schirmer score: control > 10 mm/5min, n=16; moderate ADDE 5-10 mm, n=13; severe ADDE < 5 mm, n=14. NS tear samples were collected for BioRad 27-Plex cytokine assay. At the same patient visit, 8 conjunctival impression cytology (CIC) specimens were collected per eye, RNA extracted, and RT-qPCR conducted on TaqMan 384-well/96A low density arrays.

**Results:** Significant differences were found between ADDE groups: Tear IL-6, IL-1β, IL-8, G-CSF and IP-10 correlated significantly with expression of IL-17 conjunctival genes with inflammatory roles: primarily MAPK pathway, Th17 pathway and apoptosis. All five tear cytokines correlated with complement C3, MIP-3β, IL-10α, NOS2, GM-CSF, MIG, IP-10 and TNF gene expression. All older tear cytokines including TNF-α showed few correlations. Control group: MAPK tear cytokines IL-6 and G-CSF correlated with >18 conjunctival genes, the gene profile overlapping <50% with the severe ADDE group. Tear IL-1β, IL-8, and IP-10 did not correlate with any conjunctival genes in controls, while TNF-α showed 11 gene correlations. In controls, additional tear cytokines, VEGF, IL-12, IL-17 and MIP-1α showed strong conjunctival gene association patterns similar to that of tear G-CSF for this group. The most commonly correlated genes in the control group were IFN-γ, CCR1, IP-10, IL-23A, IL-6 and FAS-ligand. Patterns were conversely absent in the moderate ADDE group.

**Conclusions:** A clear correlation between MAPK tear cytokines and conjunctival gene expression of associated factors seen in severe ADDE patients is absent in moderate ADDE. A very different association pattern emerges in controls. The fact that only tear IL-6 and G-CSF show strong associations with conjunctival gene expression in both severe ADDE and control patients suggests key, possibly regulatory, roles for these cytokines in ADDE.

**Commercial Relationships:** Roderick J. Fullard, None; Nicole M. Gayette, None; My-Tho K. Tran, None; John L. Bradley, None

**Support:** Eyesight Foundation of Alabama, NIH P30 EY003039

**Program Number:** 3684 Poster Board Number: A0198

**Presentation Time:** 3:45 PM–5:30 PM

**Topical immunomodulator use in the treatment of primary or secondary Sjogren dry eye disease patients**

**Rosen Hazarbassanov, Camila Yamazato, Danielle Miura, Jeison Barros, Jose A. Gomes.** Department of Ophthalmology and Visual Sciences, Paulista School of Medicine, Sao Paulo Hospital, Federal University of Sao Paulo, Sao Paulo, Brazil.

**Purpose:** To determine the efficacy of an immunomodulating topical medication containing 0.05% ciclosporine A (CsA), compared to a castor-oil based topical lubricant, on the treatment of dry eye disease (DED) due to primary or secondary Sjogren’s syndrome.

**Methods:** Clinical randomized, double-blind, efficacy and safety study (NCT02004067). Thirty seven patients with previously diagnosed primary or secondary Sjogren’s syndrome (SS) according to a revised version of the European criteria proposed by the American-European Consensus Group were included in the study. Participants were randomized in two groups, the first was composed by 20 patients (100% female; mean age±SD: 55.00±7.42) who were treated with CsA 0.05% (Restasis®, Allergan Inc.), while the second included 17 patients (94.1% female; mean age±SD: 50.86±12.06) who were treated with castor-oil based topical lubricant (Refresh Endura®, Allergan, Inc.). Both eye drops were preservative free and applied 3 times a day for 3 months. All patients were submitted to the following tests, for DED diagnose and follow-up: Ocular Surface Disease Index (OSDI), patient symptomatology questionnaire, best spectacle corrected visual acuity (BSCVA), biomicroscopy, Schirmer 1 test without anesthesia, fluorescein break up time (FBUT), fluorescein and lissamine green staining and impression cytology (IC) of superior and temporal conjunctiva.

**Results:** Both topical castor-oil lubricant and CsA treatments led to significant improvement in symptoms such as dryness, burning and foreign body sensation (Wilcoxon, p<0.05). However, CsA treatment improved OSDI, photophobia and blurred vision (Wilcoxon, p<0.05). Nonetheless, castor-oil treatment induced significant improvement in ocular pain and lissamine green conjunctival staining (Wilcoxon, p<0.05). IC in temporal conjunctiva did not show a significant improvement or worsening of total score after 3 months treatment with either for castor-oil or CsA (Wilcoxon, p<0.05).

**Conclusions:** Both topical CsA 0.05% and castor oil eye drops treatments are effective for the treatment of Sjogren’s DED, while IC findings show that the inflammatory process remains stable. Those findings suggest that immunomodulatory or castor-oil eye drops can be prescribed for primary or secondary Sjogren’s syndrome DED patients.

**Commercial Relationships:** Rossen Hazarbassanov, None; Camila Yamazato, Danielle Miura, Jeison Barros, Jose A. Gomes.

**Clinical Trial:** NCT02004067

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Presentation Time: 3:45 PM–5:30 PM

Neutrophil Extracellular Traps accumulate on ocular surface of Dry Eye Disease patients: Potential for a new therapeutic strategy using DNase I eye drops

Yair Ivari1, Sapna Tibrewal, Joy Sarkar, Eunjae Kim, Sarmad H. Jassim, Snehal Sonawane, Yong-Soo Byun, Rama Wahood, Lauren Schneider, Sandeep Jain.

Corneal Neurobiology Laboratory, Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, College of Medicine, Chicago, IL.

Purpose: We hypothesize that Neutrophil Extracellular Traps (NETs) and Extracellular DNA (eDNA) production and clearance mechanisms are dysregulated in Dry Eye Disease (DED). We investigated the abundance of NETs and eDNA as well as the clinical benefit of clearing them with DNase I eye drops.

Methods: Mucoid films (MF) from the ocular surface and exfoliated cells that adhered to Schirmer test strips were collected and mounted on glass slides. Immunofluorescence confocal microscopy was used to evaluate neutrophils, eDNA, NETs, and their molecular components. DNA abundance was determined in the tear fluid of patients with DED and of healthy control subjects using PicoGreen dye assay. Two patients with DED were treated with DNase I eye drops.

Results: MFs from DED patients showed presence of numerous Neutrophils and NETs admixed with epithelial cells. High infiltration of Neutrophils (>15 per 20x field) was observed in nine (64%) patients with graft versus host disease (GVHD). Larger than normal neutrophil diameter and abnormal nuclear morphology (indistinct lobes or diffuse) pointed towards activated neutrophils. PicoGreen dye assay from DED patients’ tear fluid showed two important findings. First, the fluorescence signal measurements at short incubation times (2 to 5 minutes) measured eDNA levels in. Second, the increase in fluorescence signal on kinetic assay over 20 minutes occurred due to PicoGreen dye assay buffer-induced degeneration of admixed cells and consequent release of intracellular DNA into the medium. The eDNA abundance in healthy control subjects’ tear fluid was 1.4±0.2 μg/ml. eDNA abundance in tear fluid of patients with non-autoimmune DED, autoimmune DED, and GVHD was significantly higher (2.9±0.6, 5.2±1.2, and 9.1±2.3 μg/ml, respectively; p<0.05). Treatment of DED patients with DNase I eye drops reduced eDNA abundance, abrogated signal increase, and improved comfort.

Conclusions: Our findings point to novel therapeutic interventions in severe DED based on clearance of eDNA, NETs, and other molecular components from the ocular surface. An approach for managing DED can be to measure eDNA abundance in tear fluid with the PicoGreen dye assay and reduce excessive amounts with DNase I eye drops.

Commercial Relationships: Yair Ivari, None; Sapna Tibrewal, None; Joy Sarkar, None; Eunjae Kim, None; Sarmad H. Jassim, None; Snehal Sonawane, None; Yong-Soo Byun, None; Rama Wahood, None; Lauren Schneider, None; Sandeep Jain, None.

Support: National Eye Institute (NEI) Grant EY018874 and R01EY023656 (SJ), and Research to Prevent Blindness R01EY023656 (SJ), NEI core grant EY001792, Midwest Eye Banks National Eye Institute (NEI) Grant EY018874 and.

Program Number: 3685 Poster Board Number: A0199

Program Number: 3686 Poster Board Number: A0200

Topical Interleukin-1 (IL-1) Receptor Inhibition Reduces Ocular Pain

Eric S. Furfine1, Reza Dana2, Cameron Wheeler3, Abbie Celniker4, Michael H. Goldstein1. 1R&D, Eleven Biotherapeutics, Cambridge, MA; 2Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, Boston, MA.

Purpose: Ocular pain is a common patient symptom in many ophthalmic diseases. Multiple preclinical studies indicate that IL-1β acts directly on IL-1R1 receptors on the surface of neurons throughout the body to induce hyperalgesia. IL-1 agonists are elevated and involved in the inflammation that occurs on ocular surface tissues in dry eye disease (DED), allergic conjunctivitis, LASIK surgery, photorefractive keratotomy and other ocular surface disorders. As the cornea has the densest neuroplexus in the body, blockade of elevated IL-1β in DED might reduce ocular pain and discomfort associated with DED.

Methods: EBI-005 is a novel IL-1R1 inhibitor designed and optimized for topical treatment of ocular surface inflammatory disease. A Phase 1b/2a study randomized 74 subject to topical vehicle control or EBI-005 (5 or 20 mg/mL), treated 3x/day for 6 weeks. Symptoms were assessed using the Ocular Surface Disease Index (OSDI). Of the 12 OSDI questions asked of subjects, one of the questions specifically has subjects rate their “painful or sore eyes” (OSDI-Pain score) on a scale 0 - 4 (none to frequent pain). A separate study, treated subjects with topical recombinant IL-1 receptor antagonist (IL-1Ra, same mechanism of action as EBI-005) administered three times daily for 12 weeks and assessed OSDI scores. Retrospective analyses assessed the effect of IL-1 blockade on the OSDI-Pain score by comparing change from baseline for the drug-treated and vehicle control groups over the course of the respective treatment phases.

Results: 100% of subjects randomized in both trials responded to the OSDI-Pain question. In the EBI-005 trial, subjects receiving EBI-005 (efficacy evaluable) improved from baseline by 46% (0.9 units) in OSDI-pain at 6 weeks, with some improvement as early as two weeks. OSDI-Pain improved by 61% (0.9 units) in subset of subjects with a baseline total OSDI score under 50. For the IL-1Ra trial, subjects improved from baseline by 37% and 36% in OSDI-Pain at 6 and 12 weeks. Corneal esthesiometry showed EBI-005 did not change sensation from baseline over the 6 week treatment.

Conclusions: Two separate studies of subjects with DED treated topically with two different IL-1R1 inhibitors (EBI-005 or IL-1Ra) resulted in substantial improvement in OSDI-Pain score compared to vehicle treatment. These results suggest that blockade of IL-1 agonist signaling in the eye may have direct effects on ocular pain associated with DED.

Commercial Relationships: Eric S. Furfine, Eleven Biotherapeutics (E); Reza Dana, Eleven Biotherapeutics (C); Cameron Wheeler, Eleven Biotherapeutics (E); Abbie Celniker, Eleven Biotherapeutics (E); Michael H. Goldstein, Eleven Biotherapeutics (E)

Clinical Trial: NCT01745887

Program Number: 3687 Poster Board Number: A0201

Presentation Time: 3:45 PM–5:30 PM

The brain-derived neurotrophic factor rs6265 (Val66Met) polymorphism and dry eye disease: Potential association with stress-related disorders (depression/anxiety)

Joelle Hallak1, Xiaoyi Gao2, Sandeep Jain1. 1Corneal Neurobiology Laboratory, Department of Ophthalmology & Visual Sciences, Univ Illinois Chicago, IEEI, Chicago, IL; 2Quantitative Ocular Genomics Laboratory, Ophthalmology and Visual Sciences, Univ Illinois Chicago, IEEI, Chicago, IL.

Purpose: To determine the effect of rs6265 single nucleotide polymorphism (SNP) in the Brain-Derived Neurotrophic Factor (BDNF) gene in dry eye disease (DED), and determine the association between rs6265 and stress-related disorders (depression/anxiety) in DED patients.

Methods: BDNF rs6265 genotype and allele frequency were measured using TaqMan SNP genotyping assay for 65 DED patients
and 11 controls. DED was measured through symptom and clinical examination of new and established patients. Differences in genotype frequency (G/G, G/A, and A/A) by DED status was determined using Fisher’s exact test. Among DED patients, depression and/or anxiety status was determined through patient and medication history. Logistic regression was used to determine the association between G/A genotype and depression/anxiety status.

**Results:** The G/A genotype was higher in DED as compared to controls (40% versus 10%). Sixty percent of DED patients had a genotype of G/G and 90% of controls had a genotype of G/G. This difference was not significant (P > 0.05). The minor allele frequency (A) in DED cases was 0.155 compared to 0.09 in controls. Only one patient had a genotype of A/A. Among DED, patients with the G/A genotype were 4.8 times more likely than patients with the G/G genotype to have a depression/anxiety disorder (P=0.0016).

**Conclusions:** In this preliminary data and analysis, BDNF rs6265 SNP may play a role in patients with DED and other stress-related disorders.

**Commercial Relationships:** Joelle Hallak, None; Xiaoyi Gao, None; Sandeep Jain, None

**Support:** Illinois Society to Prevent Blindness and Research to Prevent Blindness

**Program Number:** 3688 **Poster Board Number:** A0202

**Presentation Time:** 3:45 PM–5:30 PM

**Safety and Efficacy of Topical 1% docosahexaenoic acid (DHA) and 1% alpha-linoleic acid (ALA) in a Canine Model**

Mausam R. Damani1, Simone Iwabe2, Gustavo D. Aguirre2, Karla Carlisle2, Maxwell Pistilli1, Vatinee Y. Bunya2. 1Department of Ophthalmology, University of Pennsylvania, Philadelphia, PA; 2Department of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

**Purpose:** To evaluate the safety and effect on tear film cytokine levels of a topical fatty acid formulation consisting of 1% docosahexaenoic acid (DHA) and 1% alpha-linoleic acid (ALA).

**Methods:** The right eye of 10 dogs was treated with the study drug (1% DHA and 1% ALA) three times a day for 4 weeks, while the left eye was treated at the same frequency with a control vehicle. Ocular surface examinations, including portable slit lamp examination and ocular surface staining with fluorescein, were performed at baseline and weekly thereafter for a total of 6 weeks. Schirmer strips were analyzed for cytokine levels of the following cytokines: IFNγ, TNFα, IL-1β, IL-2, IL-6, IL-8 and IL-10. Conjunctival biopsies were performed at 4 weeks and mRNA levels of the same cytokines were assessed. Systemic toxicity was monitored with blood samples to evaluate the complete blood count (CBC), basic metabolic panel (BMP), and liver function tests (LFTs).

**Results:** The study drug was well tolerated by all dogs, with no signs of local erythema, irritation or inflammation. In addition, no evidence of systemic toxicity was noted on serum testing of CBC, BMP and LFTs. At 4 weeks, there was no difference in the tear film concentration of any of the studied cytokines between the treatment and control eye in any of the dogs (Table 1). Intra-eye comparisons were also done to look for changes in absolute cytokine levels throughout the course of the study. Most notable was the trend in IL-8 in the treated eye, which dropped dramatically from baseline and then slowly began to rise again after treatment was complete (P<0.01, Table 2).

**Conclusions:** We found that the combination of 1% docosahexaenoic acid (DHA) and 1% alpha-linoleic acid (ALA) was a well tolerated topical fatty acid formulation. Secondary analysis of the effects of this formulation revealed no significant difference between the treated and control eye on the absolute levels of most cytokines in the tears. However, treatment had a significant impact on decreasing IL-8 levels, a key component in the inflammatory pathway. Although safety was established, our study was not powered to detect subtle differences in cytokine levels. Larger studies to evaluate the impact of topical fatty acids on cytokine levels are needed in order to further explore the role of these substances in treating inflammation-mediated diseases such as dry eye syndrome.

**Table 1:** Comparison of cytokines, mRNA expression, and Schirmer’s test at week 4, by treatment

<table>
<thead>
<tr>
<th>Marker</th>
<th>Untreated</th>
<th>Median* (25%, 75%)</th>
<th>Treated</th>
<th>Lower</th>
<th>Same</th>
<th>Higher</th>
<th>p-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNγ</td>
<td>&lt;d (&lt;d, 65)</td>
<td>&lt;d (&lt;d, 65)</td>
<td>3 (30%)</td>
<td>7 (70%)</td>
<td>0 (0%)</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>TNFα</td>
<td>4.5 (&lt;d, 83)</td>
<td>0.97 (&lt;d, 63)</td>
<td>4 (60%)</td>
<td>4 (60%)</td>
<td>2 (20%)</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>29 (43, 44)</td>
<td>20 (&lt;d, 28)</td>
<td>5 (50%)</td>
<td>3 (30%)</td>
<td>2 (20%)</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>8.5 (&lt;d, 22)</td>
<td>2.4 (&lt;d, 28)</td>
<td>5 (50%)</td>
<td>4 (40%)</td>
<td>1 (10%)</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td>128 (134, 2263)</td>
<td>665 (&lt;d, 1961)</td>
<td>6 (60%)</td>
<td>2 (20%)</td>
<td>2 (20%)</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>&lt;d (&lt;d, 64)</td>
<td>&lt;d (&lt;d, 64)</td>
<td>0 (0%)</td>
<td>10 (100%)</td>
<td>0 (0%)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>mRNA IFNγ</td>
<td>0.021 (0.017, 0.027)</td>
<td>0.023 (0.014, 0.032)</td>
<td>5 (50%)</td>
<td>0 (0%)</td>
<td>5 (50%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>TNFα</td>
<td>0.014 (0.011, 0.021)</td>
<td>0.015 (0.010, 0.021)</td>
<td>5 (50%)</td>
<td>0 (0%)</td>
<td>5 (50%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.035 (0.023, 0.056)</td>
<td>0.034 (0.027, 0.043)</td>
<td>5 (50%)</td>
<td>0 (0%)</td>
<td>5 (50%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>0.015 (0.011, 0.023)</td>
<td>0.015 (0.009, 0.021)</td>
<td>6 (60%)</td>
<td>0 (0%)</td>
<td>4 (40%)</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td>0.029 (0.021, 0.041)</td>
<td>0.030 (0.023, 0.042)</td>
<td>7 (70%)</td>
<td>0 (0%)</td>
<td>3 (30%)</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>0.076 (0.056, 0.11)</td>
<td>0.070 (0.049, 0.085)</td>
<td>6 (60%)</td>
<td>0 (0%)</td>
<td>4 (40%)</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>0.043 (0.022, 0.045)</td>
<td>0.036 (0.025, 0.039)</td>
<td>7 (70%)</td>
<td>0 (0%)</td>
<td>3 (30%)</td>
<td>0.34</td>
<td></td>
</tr>
</tbody>
</table>

* d indicates below level of detection
** Sign test

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Commercial Relationships: Mausam R. Damani, None; Simone Iwabe, None; Gustavo D. Aguirre, None; Karla Carlisle, None; Maxwell Pistilli, None; Vatinee Y. Bunya, None

Support: K12-EY-015398, Research to Prevent Blindness

Program Number: 3689 Poster Board Number: A0203
Presentation Time: 3:45 PM–5:30 PM

Dry Eye Response to Topical Steroids: an in vivo Confocal Study

Eduardo Villani, Elena Garoli, Veronica Canton, Vittoria Termine, Roberto Ratiglia, Paolo Nucci. Clinical Sciences and Community Health, University of Milan, Milan, Italy; University Eye Clinic, San Giuseppe Hospital, Milan, Italy; Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy.

Purpose: To evaluate, in moderate to severe dry eye patients treated with topical corticosteroids, the in vivo laser scanning confocal microscopy (LSCM) corneal findings and their association with clinical response to treatment.

Methods: We consecutively recruited 50 patients with moderate to severe dry eye. Exclusion criteria were trauma or surgery in the previous 6 months, any systemic or ocular disease (other than dry eye) and any systemic or topical treatment (except artificial tears), ongoing or performed in the previous 3 months, with known effect on the ocular surface. Baseline and follow-up visits included Ocular Surface Disease Index (OSDI) questionnaire, full eye exam and LSCM study of central cornea (including superficial and basal epithelial cells density, anterior, posterior and activated keratocytes density, sub-basal dendritic cells density (DCD), and sub-basal nerves length and tortuosity). All patients were treated with loteprednol etabonate q.i.d. for 4 weeks. The follow-up visit was performed 30±2 days after the baseline. We compared clinical and confocal data obtained before and after treatment and looked for associations between baseline data and steroids-induced changes. Basing on the previously validated OSDI Minimal Clinically Important Difference, we re-analyzed the baseline findings comparing steroids responders to non-responders. Statistical analysis considered the worst eye.

Results: OSDI score and DCD significantly decreased after treatment (Paired samples t-test: 41.8±20.9 vs 52.3±20.7, P<0.01 and 64.3±45 vs 138.4±106.7 cells/mm²; P<0.01, respectively). DCD baseline values showed significant correlations with both OSDI and DCD steroid-related changes (r=0.44, P<0.05 and r=0.70, P<0.01, respectively). Baseline mean DCD was significantly higher in responders to steroids compared to non-responders (Independent samples t-test: 164.1±109.2 vs 110.8±45.5; P<0.05).

Conclusions: In vivo confocal evaluation of DCD is effective in detecting steroid-related corneal inflammation changes. DCD baseline values are associated to symptoms' improvement after treatment. These promising preliminary data suggest the need for future studies, designed to test the predictive value of DCD for clinical response to steroids treatment.

Confocal images showing DCD in a patient responder to steroids (A, B) and in a non-responder one (C, D) before (A, C) and after (B, D) 4 weeks of treatment.

Commercial Relationships: Edoardo Villani, None; Elena Garoli, None; Veronica Canton, None; Vittoria Termine, None; Roberto Ratiglia, None; Paolo Nucci, None

Support: K12-EY-015398, Research to Prevent Blindness
Objective Evaluation of Ocular Surface Lubricants in Dry Eye Patients using Thermal Imaging

Ranjini Kottaiyan1, Holly B. Hindman1, Geunyoung Yoon1, 2, Stephen Davio3, James Zavislanski3, James Aquavella1. 1Flaum Eye Institute, University of Rochester, Rochester, Rochester, NY; 2The Institute of Optics, University of Rochester, Rochester, Rochester, NY; 3Center for Visual Science, University of Rochester, Rochester, Rochester, NY; 4Bausch & Lomb, Rochester, NY.

Purpose: To objectively evaluate the effectiveness of two ocular surface lubricants in subjects with dry eyes, by studying the changes in ocular surface temperature (OST) and compare the results to a saline eye drop (control) in a double masked study.

Methods: 15 eyes of subjects clinically diagnosed with dry eye were randomized to receive two tear drops, drop A (propylene glycol and glycerin containing drop) and drop B (carbomer containing drop) and the OST changes were compared to that of a control saline drop (Ocufresh eye wash), on three separate days. The OST was measured using a non-invasive infrared thermal camera (Thermovision SC325, FLIR System, sensitivity <0.05°C at +30°C, 30HZ frame rate, 320X240 resolution, accuracy ± 2% with emissivity set at 0.98) at baseline (before drop), 5 min, 15 min and 30 min after drops. Thermal data was analyzed using custom software to calculate the initial OST, average OST and slope of OST in the central 9 mm of the cornea over a five second blink interval. Statistical analyses were performed using t-tests.

Results: No significant changes in initial OST were observed with any of the drops. With drop A, there was an increase in average OST from 5 min (34.14 ±0.15°C) to 15 min (34.46 ±0.11°C, p=0.006), and a decrease in OST from 15 min to 30 min (34.29 ±0.11°C, p=0.02) after drop instillation. With drop B, there was a decrease in the OST from baseline (34.17 ±0.13°C) to 5 min (33.8 ±0.16°C, p=0.04), returning to baseline at 15 min (34.05 ±0.17°C, NS). With saline, no significant change in average OST was observed. On comparing the study drops to saline, change in OST from baseline with drop A was greater at 5 min (-0.11°C, NS), 15 min (0.12°C, p=0.02) and 30 min (-0.13°C, p=0.01). No significant change in OST was noticed between drops B and saline. With drop A, the rate of OST cooling was lower than that in the tear. CSA levels were not detectable in aqueous humor (< 1 ng/ml) at all time points.

Conclusions: A combination of Propylene glycol and glycerin containing eye drop helps increase the OST in dry eyes better than a carbomer containing drop. This may indicate decreased evaporation with drop A. Thermal imaging helps study the effectiveness of ocular surface lubricants and has the potential for usage in the study of different dry eye treatments.

Commercial Relationships: Hai Tang, None

Clinical Trial: NCT01375582

Program Number: 3692 Poster Board Number: A0206
Presentation Time: 3:45 PM–5:30 PM

Safety evaluation of ocular drug delivery formulation: an in vivo approach

Vasudha Gupta1, Holly I. Lorentz2, Ben B. Muirhead1, Heather Sheardown1, 2. 1Ophthalmology, McMaster University, Hamilton, ON, Canada; 2Chemical Engineering, McMaster University, Hamilton, ON, Canada; 3Biomedical Engineering, McMaster University, Hamilton, ON, Canada.

Purpose: To validate a simplified liquid chromatograph coupled with tandem mass spectrometry (LC-MS-MS) method with sensitivity and specificity for determination of Cyclosporine A (CSA) in rabbit tissues and application to the pharmacokinetic studies.

Methods: A 50μl of 0.05% CSA was instilled into the left eye of 20 rabbits and the animals divided evenly into four groups. From each group, tear specimens were collected at 0.5, 2, 12, 24 hours and the animals sacrificed immediately to collect aqueous humor from the anterior chamber, and subsequently the cornea and conjunctival tissues.

CSA and internal standard (IS, Etofesalamide) were extracted from specimens by protein precipitation with methonal. Chromatographic separation was carried out on a Diamonsil C8 column with a mobile phase of methanol-water (including 25mM ammonium acetate) using gradient elution. Mass spectrometric detection was achieved by a triple-quadrupole mass spectrometer equipped with an ESI interface operating in positive ionization mode. Quantitation was performed using multiple reaction monitoring (MRM) of precursor-product ion transition at m/z 1219.8→m/z 1202.8 for CSA.

Results: The LC-MS-MS method allowed CSA quantitation as low as 1.0 ng/ml (R>0.99) with a sample run time of 10 minutes, for up to 24 hours after a single administration of 0.05% CSA to the eye. The highest concentration of CSA found was at 0.5 hour in tear (8988.8 ng/ml), and at 2 hours in cornea and conjunctiva (513.6 ng/ml and 451.2 ng/ml, respectively). The CSA levels reduced to 449 ng/ml, 420.6 ng/ml and 391.4 ng/ml at 24 hour, respectively. The elimination rates of the CSA in cornea and conjunctiva were much lower than that in the tear. CSA levels were not detectable in aqueous humor (< 1 ng/ml) at all time points.

Conclusions: Our simplified method achieved the required sensitivity and specificity for a pharmacokinetic study in rabbit specimens after a topical administration of CSA. Lack of detectable level of CSA in the aqueous humor most likely is due to the highly lipophilic nature of CSA. Corneal stroma, a highly hydrophilic layer, is an impermeable barrier to the absorption of CSA into the aqueous humor. The presence of high level CSA in tear, conjunctiva and cornea 24 hours after topical administration suggests that a once a day dosing should be adequate for the treatment of ocular surface inflammation seen in keratoconjunctivitis sicca.

Commercial Relationships: Hai Tang, None

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Program Number: 3693 Poster Board Number: A0207
Presentation Time: 3:45 PM–5:30 PM

Ocular Pharmacokinetics of P-321, a Novel Long-Acting Epithelial Sodium Channel Blocker

John H. Ansede, William Thelin, Richard C. Boucher, M. Ross Johnson, Pramod Terse, Katherine Warren, Jose L. Boyer. Parion Sciences, Durham, NC; Covance Laboratories, Madison, WI; National Institute of Health, Bethesda, MD; University of North Carolina, Chapel Hill, NC.

Purpose: P-321 is a potent epithelial sodium channel (ENaC) blocker that is being developed by Parion Sciences as a topical therapy for the treatment of dry eye. ENaC plays a key role in the regulation of tear fluid and is therefore an attractive target for the treatment of dry eye. To study the pharmacokinetics of P-321 in the eye, P-321 was administered via ocular instillation and drug concentrations in ocular tissues were measured following a single dose or during repeated administration over 14-days.

Methods: P-321 (0.1%) was administered via ocular instillation to Dutch Belted rabbits. Drug levels were measured in plasma, tears, and ocular tissues following a single administration and at different times during QID administration for 14 days. Following extraction, the concentration of P-321 was assayed by LC-MS/MS.

Results: P-321 is metabolically stable and therefore only the parent drug was monitored. Following single administration, P-321 elimination from tears was biphasic showing a rapid initial loss from tears followed by a long terminal elimination phase (t1/2 = 24 hr). Upon repeated administration, tear concentrations of P-321 increased and reached steady state levels by Day 6. P-321 was retained in the conjunctiva following single dose administration with palpebral drug concentration approximately five times higher than bulbar conjunctiva. After a single dose, conjunctiva concentrations of P-321 remained relatively constant for up to 48 hours and steady-state levels were achieved by Day 2 during 14 days of QID dosing. P-321 was only detected at sub nanomolar concentrations in aqueous humor five minutes post-dose. Aqueous humor concentrations increased slightly during multiple dose administration reaching steady state levels by Day 4. P-321 was detected in the cornea immediately following dose administration, however, no drug was detected thereafter or during 14-days of QID administration. P-321 was not detected in retina or lacrimal glands.

Conclusions: P-321 is a novel ocular hydrating agent capable of restoring normal tear volume in a dry eye animal model. The results presented in this study indicate that P-321 is well retained on the ocular surface of the eye (primarily in the tears and conjunctiva), providing the pharmacokinetic basis to explain its long duration of action. Furthermore, P-321 shows very little penetration or accumulation in other ocular tissues.

Commercial Relationships: John H. Ansede, Parion Sciences (E); William Thelin, Parion Sciences (E); Richard C. Boucher, Parion Sciences (C); M. Ross Johnson, Parion Sciences (E); Pramod Terse, None; Katherine Warren, None; Jose L. Boyer, Parion Sciences (E)

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Program Number: 3694 Poster Board Number: A0208
Presentation Time: 3:45 PM–5:30 PM

Effectiveness of an eyelid thermal pulsation procedure to treat recalcitrant dry eye symptoms after refractive surgery

Craig Schallhorn, Steven C. Schallhorn, Julie M. Schallhorn. 1University of California, San Diego, San Diego, CA; 2UCSF, San Francisco, CA.

Purpose: To provide an initial retrospective evaluation of the effectiveness of a thermal pulsation system to treat post-refractive surgery dye eye refractory to conventional therapy.

Methods: A total of 169 eyes of 91 patients underwent thermal pulsation therapy (LipiFlow, Tear Sciences, Morrisville, NC) for the treatment of post-operative dye eye that were not well controlled with conventional treatment. Pre-therapy measurements were conducted on the tear film lipid layer (Lipi score) and meibomian gland function (MFE). A standard dry eye questionnaire was administered to all patients before and after thermal pulse therapy to evaluate dry eye symptoms (SPEED II).

Results: The mean patient age was 51 ± 13.0 yrs, 66% were female, and their primary refractive procedure was either laser vision correction (72% LASIK / 11% PRK) or a refractive lens exchange (17%). They underwent thermal pulsation therapy a mean of 43.8 ± 23.0 months after their primary procedure. Pre-therapy Lipi score was 68.2 ± 23.4 OD, 70.6 ± 22.6 OS. Pre-therapy MGE was 4.4 ± 2.0 OD, 4.8 ± 2.1 OS. Mean pre-therapy SPEED II questionnaire score was 17.6 ± 7.6, with a reduced mean post-therapy score of 9.4 ± 5.1 (P<0.001). This statistically significant reduction in SPEED II questionnaire score correlated with an improvement in dry eye clinical findings. Symptoms were reported as “improved” in 42% of patients, with symptoms being reported as “worse” in 8%. Fifteen patients (27 eyes) reported that the effect of the therapy had worn off at a mean post-therapy time of 12.8 ± 12.0 weeks.

Conclusions: Management of recalcitrant dry eye after refractive surgery is often challenging with limited treatment options currently available. In this initial retrospective evaluation, a significant improvement in patient reported dry eye symptoms (mean pre to post-therapy change of 8.2 points) was observed following thermal pulsation therapy. However, within 6 months of treatment, the effect diminished in a subset of patients. Additional studies are needed to evaluate this treatment modality, including prospective, masked studies with a control population as well as an evaluation of the long-term efficacy.

Commercial Relationships: Craig Schallhorn, None; Steven C. Schallhorn, Abbott Medical Optics (C); Julie M. Schallhorn, None
Purpose: Hydroxypropyl Guar (HPG) in the presence of demulcients propylene glycol and polyethylene glycol has been shown to provide cellular protection from desiccation. This work describes pre-clinical data to show the benefits of a dual polymer system comprised of Hydroxypropyl Guar and Hyaluronic acid (HA) in providing prolonged protection, retention of effect and recovery from damage in human corneal epithelial (HCE) cell models.

Methods: The ability of the dual polymer solution to protect human corneal epithelial cells from desiccation was tested in a temperature/humidity controlled environment. Cells were incubated with test solutions for 30 minutes and then desiccated for 0 or 30 minutes at 37°C, 45% relative humidity. To assess retention of cellular protection, cells were washed between test compound exposure and desiccation multiple times. A cell proliferation assay was used to determine % protection compared to controls. HCE cells were exposed to triton-X-100 for 30 minutes following initial exposure with test formulations to produce damage. Ability of the test formulations to provide barrier protection and promote cellular recovery was examined using sodium fluorescein permeability (NaF) and trans-epithelial electrical resistance (TEER) assays.

Results: The dual polymer combination demonstrated significantly greater hydration protection (54%) and substantivity (50%) than HPG (37% and 30%) and HA (15% and 10%) alone (p<0.05). There was a statistically significant reduction in % fluorescence (p<0.05) when the HCE cells were allowed to recover from damage after treatment with the combination system (69%) relative to HPG (93%) and HA (83%) alone. The lower % fluorescence value is indicative of improved barrier protection, cellular recovery and regeneration.

Conclusions: The combination of HPG/HA with the active demulcients provides an effective lubricant formulation that shows prolonged hydration protection and recovery in response to external stress environments in an in-vitro HCE cell model. These data provide supporting evidence for the dual polymer solution in potentially promoting desiccation protection and retention on the ocular surface.

Commercial Relationships: Rekha Rangarajan, Alcon Laboratories, Inc. (E); Brian Kraybill, Alcon Laboratories, Inc. (E); Howard A. Ketelson, Alcon Laboratories, Inc. (E)
Disabling Single Use Ophthalmic Medical Devices Sterilized with Gamma Radiation

Jeffery Rosino, Jordan Hutchinson, Stephen Grenon. TearScience, Inc., Morrisville, NC.

Purpose: Disposable ophthalmic medical devices, at times, need to be disabled to prevent re-use. Using electronic components to disable a medical device can be problematic if gamma radiation sterilization is utilized during the manufacturing process. This report tests the effects of gamma radiation on two methods of disabling a disposable medical device, a simple surface mount fuse and a novel Ferroelectric Random Access Memory (FRAM). The FRAM has the advantage of being able to record treatment data as well as disable the device after use.

Methods: Ten circuit boards were built each containing an FM24V10 FRAM by Ramtron. One hundred circuit boards populated with 125mA surface mount fuses and 100 circuit boards populated with 250mA surface mount fuses were also built. The FRAM devices were completely programmed to a preset repeating value. The fuses were measure and shown to be connected and working to specification. Gamma sterilization was performed per ANSI/AAMI/ISO 11137-1:2006 at the 25 kGy level. Following Gamma radiation sterilization the FRAM memory devices content was compared to the pre sterilization state. The FRAM memories were further exercised by reading and writing to all the memory locations to show proper functionality. The fuses were also measured and compared to their pre-radiation state.

Results: The FRAM circuits demonstrated no sign of performance degradation. The content of all one million memory locations remained intact after gamma radiation of 28 kGy and the circuit was able to read and write information to all memory locations. Two of the 100 fuses rated at 125 mA were destroyed as demonstrating by an open circuit, indicating a 2% failure rate. None of the 100 fuses rated at 250mA were destroyed.

Conclusions: The FM24V10 FRAM memory devices can be programmed prior to gamma sterilization with an expectation that the data will not be corrupted by the gamma radiation sterilization process. Furthermore, the FRAM memory device will continue to function normally. However, if a fuse is used to disable the medical device, care should be taken to size the fuse large enough so that eddy currents induced during gamma radiation sterilization do not destroy the fuse.

Commercial Relationships: Jeffery Rosino, TearScience, Inc. (E); Jordan Hutchinson, TearScience, Inc. (E); Stephen Grenon, TearScience, Inc. (E)