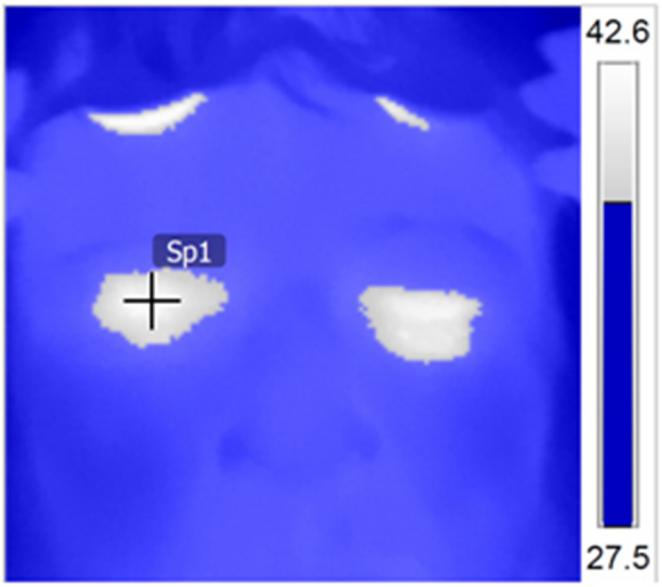
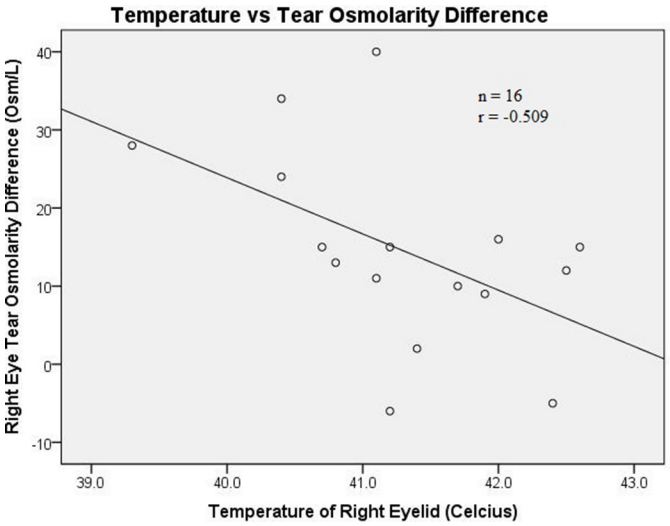
133 Dry eye, non-clinical
Sunday, May 07, 2017 1:30 PM–3:15 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 441–499/A0366–A0424
Organizing Section: Cornea

Program Number: 441 **Poster Board Number:** A0366
Presentation Time: 1:30 PM–3:15 PM
Anti-inflammation effects of Zidovudine in human corneal epithelial cells
hui liu[1, 2], Frank Gambino[3], Taylor Keller[3], Yougang Zhai[3], Liang Qiao[3], Charles S. Bouchard[1], Ping Bu[1], Shaozhen Zhao[2].
[1]Ophthalmology, Loyola University Chicago, Maywood, IL; [2]Tianjin Medical University Eye Hospital, Tianjin, China; [3]Microbiolology & Immunology, Loyola University Chicago, Maywood, IL.
Purpose: Dry eye disease (DED) is a common ocular surface inflammatory disease, which can significantly impact the quality of life of patients. Activation of NLRP3 inflammasome and downstream casepase-1 and IL-1β activation and maturation play critical roles in the pathophysiology of DED. The purpose of this study is to determine the effect of Zidovudine (AZT), a nucleoside reverse transcriptase inhibitor, on inflammasome activation in corneal epithelial cells, a hyperosmolarity dry eye model.
Methods: Immortalized human corneal epithelial cells (HCECs) were pretreated in the presence or absence of 100mM and 200mM of AZT for 2 hours. HCECs were then exposed to media ranging from 312 mOsm (normal osmolarity) to 500 mOsm (hyperosmolarity). Cell culture supernatants were collected at 4, 12, and 24 hours. Epithelial cell viability and level of IL-1β were determined by a lactate dehydrogenase (LDH) assay and a specific capture ELISA (eBioscience).
Results: In the hyperosmolarity group, the LDH ratio and level of IL-1β were significantly increased as compared to the normal control osmolarity group. In the hyperosmolarity group pretreated with 100mM and 200mM AZT, the LDH ratio and IL-1β level were decreased as compared to non-AZT-treated hyperosmolarity group.
Conclusions: AZT can improve HCECs survival as evidenced by decreased LDH ratio and inhibit the production of the inflammatory cytokines IL-1β. Nucleoside reverse transcriptase inhibitors like AZT may be useful for the management of DED.
Commercial Relationships: hui liu; **Frank Gambino**, None; **Taylor Keller**, None; **Yougang Zhai**, None; **Liang Qiao**, None; **Charles S. Bouchard**, None; **Ping Bu**, None; **Shaozhen Zhao**, None
Support: This work was supported by a grant from the Illinois Society for the Prevention of Blindness (ISPB), and funding from The Richard A. Perritt Charitable Foundation.

Program Number: 442 **Poster Board Number:** A0367
Presentation Time: 1:30 PM–3:15 PM
Eyelid Temperature and Tear Osmolarity
Benjamin Lee[1], Misha Faustina[2]. [1]Meharry Medical College, School of Medicine, Nashville, TN; [2]Phoenix Oculoplastic Consultants, Phoenix, AZ.
Purpose: The effect of direct non-pulsatile external eyelid heat application on tear osmolarity has not previously been documented. We performed a retrospective cohort study to quantify how different eyelid temperatures achieved from externally applied heat may change patient tear osmolarity.
Methods: Clinic charts of 16 patients with dry eye syndrome treated with externally applied eyelid heat were retrospectively reviewed. None of the patients had known lacrimal dysfunction. According to clinic treatment protocol, patients' baseline tear osmolarity in the right eye was measured using the TearLab® Osmolarity System. Patients then received 10 minutes of direct non-pulsatile externally applied heat on the eyelids with the Digital Heat™ Precision Heated Eye Pad. After 10 minutes, the heated eye pad was removed with eyelids remaining closed to obtain a non-contact spot temperature measurement using the FLIR i7™ Thermal Imaging Camera. Following, patients opened their eyes for the immediate measurement of post-heat tear osmolarity in the right eye.
Results: Eyelids had temperature measurements ranging from 39.3 C° to 42.6 C° as a result of externally applied heat. Using a paired two sample t-test, the right eye of patients exhibited significantly lower mean tear osmolarity after 10 minutes of applied heat (319.25±16.36 Osm/L vs 304.69±11.36 Osm/L in baseline and post-heat tears, $p<0.001$ for two tail) with Cohen's size effect ($d=1.03$, 95% CI=0.29-1.77). The mean arithmetic tear osmolarity difference between tear osmolarity at baseline and post-heat was 14.56±12.47 Osm/L. Furthermore, Pearson correlation coefficient showed a significant negative correlation between the temperature measured on the right eyelid at 10 minutes and the associated arithmetic tear osmolarity difference ($r=-0.509$, $p=0.044$ for two tail).
Conclusions: Applying heat on the eyelid for 10 minutes resulted in various levels of achieved eyelid temperatures that decreased tear osmolarity. The associated large Cohen's effect size supports this finding. The negative correlation finding suggests that higher achieved eyelid temperatures may result in smaller decreases in tear osmolarity. Taken as a whole, there probably exists an optimal eyelid temperature range achieved from externally applied heat on the eyelids and length of treatment time to most effectively lower tear osmolarity. These are topics for further evaluation.



Sample of thermal image immediately following removal of Heated Eye Pad.



Commercial Relationships: Benjamin Lee, None; **Misha Faustina**, Digital Heat Corporation (C)

Program Number: 443 **Poster Board Number:** A0368
Presentation Time: 1:30 PM–3:15 PM
Rapamycin Prevents Endoplasmic Reticulum Stress-induced Dry Eye Syndrome in Mice
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Purpose: To investigate whether rapamycin protect the tear and ocular surface against the endoplasmic reticulum (ER) stress-induced dry eye syndrome in mice.
Methods: Tunicamycin was injected intraperitoneally in balb/c mice without or with rapamycin (TM group or RM5 group). Phosphate buffered saline was injected intraperitoneally in control group. Blinking rate, fluorescein staining score (FSS) and phenol red-thread tear secretion test were measured at 4 days, 1 week, and 2 weeks after treatment. Levels of inflammatory and angiogenetic cytokines were measured by ELISA.
Results: Blinking rate and FSS were elevated in TM group compared to control and tear secretion was decreased in TM group compared to control ($p<0.05$ for all), which was ameliorated by rapamycin at 1 week and at 2 weeks. Levels of inflammatory and angiogenetic cytokines in ocular surface and lacrimal glands were elevated in TM group compared to control and decreased in RM5 group compared to TM group at 1 week and at 2 weeks ($p<0.05$ for all).
Conclusions: Intraperitoneal injection of tunicamycin induced dry eye syndrome in mice. Rapamycin protected the tear and ocular surface against the ER stress-induced dry eye syndrome in mice through ameliorating ER stress-induced vascular damage and inflammation of lacrimal glands and ocular surface.
Commercial Relationships: Sang wook Choi, None; **Bum Joo Cho**, None; **Kim Jeong Won**, None; **Tae-Young Chung**, None; **Joon-Young Hyon**, None; **Young Joo Shin**, None

Clinical Trial: www.nrf.re.kr/nrf_eng_cms, NRF-2015R1D1A1A09058505

Program Number: 444 **Poster Board Number:** A0369
Presentation Time: 1:30 PM–3:15 PM
mRNA expression profile on conjunctival cells in dry eye patients using the NanoString nCounter assay system
Karima Kessal[1, 2], Hong Liang[1, 2], Ghislaine Rabut[2], Philippe Daull[3], Jean-Sebastien Garrigue[3], Mylène DOCQUIER[6], Stéphane MELIK PARSADANIANTZ[1, 4], Christophe Baudouin[1, 2], Françoise Brignole-Baudouin[1, 5]. [1]Paris, INSERM, Paris, France; [2]CIC-PSO 1423, Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, Paris, France; [3]R&D, SANTEN SAS, Evry, France; [4]CNRS, UMR_7210, Paris, France; [5]Faculté de Pharmacie, Université Paris Descartes, Paris, France; [6]iGE3 Genomics Platform, University of Geneva, Geneva, Switzerland.
Purpose: A variety of inflammatory mediators have already been reported to be involved in dry eye (DE) using classical detection methods. The NanoString's nCounter™ analysis system offers a powerful multiplexing tool to assess rapidly the gene expression of a wide range of mediators directly at the cell levels We investigated the interest of this method to detect mRNA signature in conjunctival imprints (CI) of inflammatory proteins and transcripts known to be modulated in DE patients.
Methods: Conjunctival cells were collected by CI in 15 healthy patients and 30 DE patients defined by a set of clinical signs and symptoms. Total RNAs were extracted and NanoString's nCounter™ technology with an inflammatory human Code Set was used to analyze the genes of 34 proteins. The fold change of mean with a cut-off ≥1.5 was chosen to select the differentially expressed genes in both groups. Mann-Whitney non-parametric statistical test was used.
Results: Of these 34 genes tested by the code set, 21 genes were detected. Up-regulated genes expressed with a fold change ranging from 1.4 to 16.7 such as IL-6, HLA-DRA, HLA-DRB, CXCL9, CXCL10, CCL4, CCL5, AREG, STAT1, IFIT3 and CCR1, clearly distinguished between SS patients and normal subjects, whereas IL-8, TNFα, CCL3, IFNg, TLR4, NR3C1, TGFb, ALOX15, MYC and TLR5 did not show any significant difference between groups. The remaining genes including CCL2, CXCL5, IL-1a, IL-1b, IL-2, IL-4, IL-12, IL-13, IL-17, IL-21, TGFb2, TLR9 and MMP9 were not detected.
Conclusions: These up-regulated genes may play an important role in the altered gene expression in the conjunctival epithelium and provide valuable insight into biological mechanisms underlying the pathogenesis of DE. The NanoString's nCounter™ analysis system that directly quantifies mRNA copies could detect targets identified by conventional proteomic and transcriptomic methods, and highlight multiplexing possibilities of this technology.
Commercial Relationships: Karima Kessal, SANTEN SAS (R); **Hong Liang**, SANTEN SAS (P); **Ghislaine Rabut**, None; **Philippe Daull**, SANTEN SAS (E); **Jean-Sebastien Garrigue**, SANTEN SAS (E); **Mylène DOCQUIER**, None; **Stéphane MELIK PARSADANIANTZ**, None; **Christophe Baudouin**, SANTEN SAS (C), Allergan (C), Théa (C), Alcon (C); **Françoise Brignole-Baudouin**, None

Program Number: 445 **Poster Board Number:** A0370
Presentation Time: 1:30 PM–3:15 PM
Effect of a low concentration of desonide disodium phosphate in a murine model of dry eye
Cristina Zappulla[1], Christian Scifo[1], Giuseppe De Pasquale[1], Francesco Giuliano[1], Maria Grazia Mazzone[2]. [1]Research and Preclinical Development, S.I.F.I. S.p.A., Lavinaio-Aci S.Antonio, Italy; [2]Business and Portfolio Development, S.I.F.I. S.p.A., Lavinaio-Aci S. Antonio, Italy.
Purpose: We have previously found that desonide disodium phosphate (DES) at low concentrations (i.e. 0.025%) appears to retain a significant anti-inflammatory activity on the ocular surface while sparing intraocular structures in a model of endotoxin-induced uveitis. Here, we sought to challenge our previous findings and investigated whether a formulation of xanthan gum (XNT) 0.2% containing DES 0.025% could reduce corneal damage in murine model of dry eye.
Methods: Thirthy-two C57BL/6 female mice (8–12 w) were subjected for 7 days to a controlled environment with low humidity (20% RH) and constant airflow (20 l/min). A scopolamine patch (0.75 mg) was applied to the animals' tails and renewed after 3 days. Naïve animals (CTRL-) were kept in standard housing conditions (50%–60% RH). Of the animals inside the chamber, positive control animals (CTRL+) were left untreated while the remaining groups received 10 µl q.i.d of eyedrops containing either 0.2% XNT alone or XNT 0.2% plus DES 0.025%. Fluorescein staining of the cornea was evaluated by slit lamp with a standardized grading system at baseline (t = 0) and after 3 and 7 days of treatment. Data represent the mean±s.d. of scores assigned to 8 mice per group. Statistics were by one-way ANOVA plus Bonferroni post-test.
Results: Observations at day 3 showed that corneal staining of CTRL+ mice (5.7±2.3) was significantly worsened ($p<0.001$) with respect to CTRL- (1.5±1). Treatment with XNT 0.2% did not produce any remarkable amelioration over CTRL+, while XNT 0.2% plus DES 0.025% significantly reduced corneal score to 3.5±1.7 ($p<0.05$, vs CTRL+). Observations at day 7 showed that corneal scores of CTRL+ mice remained mostly unchanged in comparison to day 3. Conversely, in mice treated with XNT 0.2% either alone or in combination with DES 0.025% corneal damage was significantly reduced by 44±17 ($p<0.05$) and 69±18 ($p<0.01$), respectively.
Conclusions: Eyedrops containing XNT were shown to reduce corneal damage caused by iposecretive/evaporative stress in a model of dry eye in mice. Notably, the addition of DES 0.025% to XNT eyedrops improved the effect exerted by XNT alone by producing an earlier onset of the protective action on corneal epithelium. These findings support the hypothesis that a low concentration of DES may have ancillary action to that of XNT in the treatment of inflammatory ailments of the ocular surface.
Commercial Relationships: Cristina Zappulla, S.I.F.I. S.p.A. (E); **Christian Scifo**, S.I.F.I. S.p.A. (E); **Giuseppe De Pasquale**, S.I.F.I. S.p.A. (E); **Francesco Giuliano**, S.I.F.I. S.p.A. (E); **Maria Grazia Mazzone**, S.I.F.I. S.p.A. (E)

Program Number: 446 **Poster Board Number:** A0371
Presentation Time: 1:30 PM–3:15 PM
CD147 and extracellular Cyplophilin A form a complex in dry eye tear film
Meredith Stallone, Sonal Sathe, Tracy T. Nguyen. Graduate Center for Vision Research, SUNY College of Optometry, New York, NY.
Purpose: Previous data collected in our lab showed that secreted CD147 (aka extracellular matrix metalloproteinase inducer, EMMPRIN) and eCyPA (extracellular cyclophilin A) are upregulated in the tear film of subjects with dry eye inflammation. CD147 has been identified as the primary receptor for extracellular cyclophilins. This interaction has been shown to be proinflammatory due to increased chemotactic activity. Inhibiting CD147 and CyPA interaction using CD147 monoclonal antibodies reduced inflammation by 50% in acute and chronic lung inflammation and rheumatoid arthritis. The purpose of this study was to establish whether CD147 and eCyP form a protein complex in the tear film of normal subjects and subjects with dry eye syndrome (DES).
Methods: Tear samples were collected from both normal and dry eye subjects using either glass microcapillaries or cotton threads. Ocular Surface Disease Index (OSDI) Questionnaire, corneal fluorescein staining, lissamine green conjunctival staining, and tear secretion with cotton threads were used to diagnose subjects with DES. Immunoprecipitation was performed using Dynabeads® Protein G and CD147 antibody. The elutant protein was analyzed by Western blotting using anti-CyPA and CyPB antibodies.
Results: Extracellular Cyclophilin A formed a complex with CD147 in 3 tear samples collected from a dry eye subject. In contrast, no CD147-eCyPA complex was found in the 3 tear samples of a normal subject. Cyclophilin B and CD147 did not associate in tear samples of either the normal or dry eye subject.
Conclusions: CD147 and CyPA form a complex in tear samples of a dry eye but not normal subject. Processing of more tear samples are needed to confirm this finding. However, preliminary results suggest that CD147-eCyPA interaction plays a role in the pathogenesis of dry eye inflammation. Inhibiting this association may provide a therapeutic option in treating dry eye inflammation. Further research is needed to understand the signaling transduction pathway initiated by CD147-eCyPA interaction.
Commercial Relationships: Meredith Stallone; **Sonal Sathe**, None; **Tracy T. Nguyen**, None
Support: None

Program Number: 447 **Poster Board Number:** A0372
Presentation Time: 1:30 PM–3:15 PM
High-mobility box group 1 protein: An alarmin driving dry eye inflammation?
Carolina Lema, Rose Y. Reins, Betty Zhang, Rachel L. Redfern. College of Optometry, University of Houston, Houston, TX.
Purpose: Dry eye disease (DED) is characterized by increased tear osmolarity and ocular surface damage due to chronic inflammation. Following cell injury or death high-mobility group box 1 (HMGB1) protein, an alarmin, is released and activates the innate immune response. Previously, we have reported that HMGB1 expression is increased in the tears of dry eye patients. Therefore, we sought to determine if HMGB1 expression increases with *in vitro* hyperosmolar stress (HOS) or in an established mouse model of experimental dry eye (EDE), thereby propagating the cycle of inflammation on the ocular surface.
Methods: Human telomerase corneal epithelial (hTCEpi) cells were treated with hyperosmolar media to induce HOS (450mOsM, 6h) or tumor necrosis factor alpha (TNF-α, 20ng/ml, 24h). Following treatment, HMGB1 expression and localization was examined by immunostaining. Alternatively, hTCEpi were stimulated with rhHMGB1 (10-1000ng/ml for 6 and 24h) and IL-6, IL-8 and TNF-α mRNA and protein were examined by qRT-PCR and ELISA, respectively. Differentiated U937 macrophages were also treated with rhHMGB1 (10ug/ml, 8h) and TNF-α secretion was measured in supernatants, to verify its biological activity. Finally, HMGB1 expression was examined in frozen tissue sections and cell lysates collected from EDE (subcutaneous scopolamine and environmental stress) and untreated (UT) C57BL/6 mice by immunostaining and qRT-PCR.

Results: HOS and TNFα induced nucleus to cytoplasm translocation of HMGB1 in hTCEpi cells. rhHMGB1 stimulation of differentiated U937 macrophages increased TNF-α protein secretion when compared to the untreated control (298±6pg/ml vs 1056±113pg/ml; p-value≤0.05). However, rhHMGB1 treatment did not significantly increase the mRNA or protein levels of IL-6, IL-8 or TNF-α in hTCEpi cells. In the mouse corneal epithelium, EDE increased HMGB1 staining and mRNA expression (1.52±0.27 vs 3.38±0.55; p-value≤0.05) compared to UT animals.
Conclusions: HMGB1 levels are increased by HOS (*in vitro*) and desiccation stress (*in vivo*). However, rhHMGB1 stimulation (*in vitro*) does not trigger inflammatory cytokines IL-6, IL-8 and TNF-α. Whether HMGB1 is directly related to inflammation in DED remains unclear and deserves further studies.
Commercial Relationships: Carolina Lema, None; **Rose Y. Reins**, None; **Betty Zhang**, None; **Rachel L. Redfern**, None
Support: NIH/NEI Grant EY023638

Program Number: 448 **Poster Board Number:** A0373
Presentation Time: 1:30 PM–3:15 PM
Cathepsin S can alter the expression of pro-inflammatory cytokines, proteases, and protease activated receptor associated with inflammatory dry eye in human corneal epithelial cells
Wannita Klinngam[1], Maria Edman[2], Zhen Meng[1], Sarah Hamm-Alvarez[2]. [1]Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA; [2]Department of Ophthalmology, Roski Eye Institute and Keck School of Medicine, University of Southern California, Los Angeles, CA.
Purpose: Cathepsin S (CTSS) has increased activity in tears of male NOD mice, a murine model of Sjögren's Syndrome (SS), and is also increased in tears of patients with SS. However, the contribution of its increased activity in tears to the ocular surface inflammation that is a part of SS has not been investigated. We hypothesized that elevated CTSS in tears might drive ocular surface inflammation by altering gene and protein expression of factors linked to corneal inflammation
Methods: The human corneal epithelial cell line (HCE-T) was cultured and treated with recombinant human CTSS at activity levels seen in SS patient tears. Gene expression of pro-inflammatory cytokines, proteases, and protease-activated receptor-2 (PAR-2) were measured after 2-,4-,8-, and 24-hours of treatment by RT-and q-PCR. CTSS protein expression and its activity in lysates were determined using Western Blotting and CTSS activity assay kits, respectively. PAR-2 protein expression was measured by immunofluorescence.
Results: Recombinant human CTSS induced gene expression of IL-6, IL-8, TNF-α and IL-1β at 2 and 4 hours. Increased IL-1β and TNF-α reached a peak at 2 hours (Relative Quantity (RQ)=2, p≤0.001 and ≤0.05 respectively). Increased IL-8 and IL-6 reached a peak at 4 hours (RQ=4, p≤0.001). CTSS, MMP-9, and PAR-2 gene expression was increased after 24-hour treatment (RQ= 4, 2, and 1.5 respectively, p≤0.001). Exposure of HCE-T cells to heat-inactivated CTSS was not as effective as in elevating IL-6 and IL-8 gene expression, suggesting that CTSS protein activity was important in eliciting the effect. Recombinant human CTSS exposure also increased expression of endogenous CTSS protein levels and endogenous CTSS activity (fold change=3.5, p≤0.05) after 24 hrs. Finally, recombinant human CTSS increased PAR-2 immunofluorescence after 24 hours.
Conclusions: These findings suggest that elevated CTSS levels in tears of SS patients may induce inflammatory cytokines, proteases, and PAR-2 expression in corneal epithelial cells, possibly contributing to ocular surface inflammation in SS patients.
Commercial Relationships: Wannita Klinngam; **Maria Edman**, None; **Zhen Meng**, None; **Sarah Hamm-Alvarez**, None
Support: NIH Grant EY011386 and Unrestricted grant from RPB

Program Number: 449 **Poster Board Number:** A0374
Presentation Time: 1:30 PM–3:15 PM
Ocular distribution and pharmacokinetics of lifitegrast following repeat topical ocular dose administration to pigmented rabbits
Jou-Ku Chung[1], Elizabeth Spencer[2], Matthew Hunt[2], Devin Welty[1], Thomas McCauley[3]. [1]Drug Metabolism and Pharmacokinetics, Shire, Lexington, MA; [2]Drug Metabolism, Covance Laboratories Inc., Madison, WI; [3]Research and Development, Shire, Lexington, MA.
Purpose: To assess ocular distribution and pharmacokinetics (PK) after repeat topical ocular administration of two Phase 3 formulations of lifitegrast in pigmented rabbits. Lifitegrast is a lymphocyte function-associated antigen-1 (LFA-1) antagonist recently approved in the US for treatment of signs and symptoms of dry eye disease (DED).
Methods: Female pigmented rabbits received a single topical ocular dose of lifitegrast (formulation #1, n=25; #2, n=25) in each eye, twice daily for 4 days and once on day 5, at a target dose level of 1.75 mg/eye/dose. Animals were euthanized on day 5; blood and ocular tissues were collected from 5 animals per formulation per time point at 0.25, 0.5, 1, 3, and 8 h after last dose. Liquid chromatography tandem mass spectrometry was used to measure lifitegrast concentrations. PK analyses (non-compartmental) included determination of maximum concentration (C_{max}), time to maximum concentration (t_{max}), and area under concentration-time curve from 0 to 8 h (AUC_{0-8}).
Results: C_{max} and AUC_{0-8} for ocular tissues and plasma were similar between the formulations. Lifitegrast concentrations were highest in the ocular anterior segment tissues, with C_{max} for the conjunctiva (palpebral and bulbar), cornea, and sclera (anterior) in the range 5190–14200 ng/g. Concentrations were lower in other ocular tissues, with the next highest C_{max} 826 ng/g for the sclera (posterior). Very low/non-detectable lifitegrast concentrations (0–36.0 ng/g) were observed in the lens, optic nerve, retina and vitreous humor. Across all tissues, t_{max} was ~0.25–1 h, indicating rapid absorption after administration. Overall exposure (AUC_{0-8}) for both formulations was highest in the conjunctiva (palpebral), followed by the cornea, sclera (anterior), conjunctiva (bulbar), sclera (posterior), iris-ciliary body, aqueous humor, and choroid-retinal pigmented epithelium. The plasma had low lifitegrast concentrations (C_{max}: formulation #1, 17.4 ng/mL; #2, 9.52 ng/mL), and t_{max} (#1 and #2, 0.25 h).
Conclusions: The high exposure of lifitegrast in rabbit ocular anterior segment tissues, and low exposure in posterior segment tissues and plasma, suggests that lifitegrast is likely to reach the target tissues for DED treatment while having low potential for off-target systemic or ocular effects. The PK profile of lifitegrast was similar between formulations.
Commercial Relationships: Jou-Ku Chung, Shire PLC (E), Shire PLC (I); **Elizabeth Spencer**, Covance Laboratories Inc. (E), Shire PLC (C); **Matthew Hunt**, Covance Laboratories Inc. (E), Shire PLC (C); **Devin Welty**, Shire PLC (E), Shire PLC (I); **Thomas McCauley**, Shire PLC (E), Shire PLC (I)
Support: Shire Development LLC

Program Number: 450 **Poster Board Number:** A0375
Presentation Time: 1:30 PM–3:15 PM
Studies on Transient Receptor Potential Vanilloid (TRPV) in human conjunctival epithelium
Dhruva Bhattacharya[1], Mingwu Wang[1, 2], Mohammad Shahidullah[1, 3]. [1]Ophthalmology and Vision Science, University of Arizona, Tucson, AZ; [2]Cornea Associates, Tucson, AZ; [3]Department of Physiology, University of Arizona College of Medicine, Tucson, AZ.
Purpose: TRPV cation channels are osmo-mechano-and-thermo-sensitive membrane proteins. These proteins are extensively involved in pathophysiology including inflammation and pain. Hyperosmotic tears and ocular surface inflammation are hallmarks of dry eye condition. Here we explore expression and osmotic modulation of TRPV1 and TRPV4 in human conjunctival epithelial cells (hCjE).
Methods: hCjE cells were cultured to confluence in supplemented Keratinocyte Serum Free Medium (GIBCO). Presence of TRPV mRNA and proteins in hCjE were determined by Reverse Transcription-Quantitative-Polymerase Chain Reaction (RT-qPCR) and western blot analysis, respectively.
Results: The RT-qPCR and western blot studies confirm the presence of TRPV1, TRPV2 and TRPV4 mRNA and proteins in hCjE cells. None of the TRPC genes were detected in the hCjE cells. Incubation of hCjE cells for 24h with 5 μM GSK 1016790A (a specific TRPV4 agonist) upregulated the TRPV4 gene by 58 folds (SEM=0.23, N=3, P=0.0001) compared to control cells. Similarly, treatment of hCjE cells with 5 μM capsaicin for 24h showed 2.6 folds (SEM=0.27, N=3, P = 0.0001) increase in TRPV1 mRNA expression compared to control cells. When cells were incubated in hyperosmotic medium (350mOsM) for 24h, TRPV1 protein level was significantly upregulated (N=3, P = 0.0008).
Conclusions: Expression of TRPV1, TRPV2 and TRPV4 and their regulation by agonists and hyperosmotic conditions in cultured hCjE signify functional presence and possibility of using this cell as an experimental platform for studying TRPV proteins under simulated dry eye condition.
Commercial Relationships: Dhruva Bhattacharya, None; **Mingwu Wang**, None; **Mohammad Shahidullah**, None
Support: ADHS14-082988

Program Number: 451 **Poster Board Number:** A0376
Presentation Time: 1:30 PM–3:15 PM
The protective effect of a topical mucin secretagogue on the ocular surface damage induced by airborne black carbon exposure
Jong Suk Song, Boram Kang, Xiangzhe Lee, Youngsub Eom, Hyo Myung Kim. Ophthalmology, Korea University College of Medicine, Seoul, Korea (the Republic of).
Purpose: Exposure to airborne particulate matter can induce ocular surface damage and inflammation. We evaluated the effects of a topical mucin secretagogue on the protection of ocular surface damage induced by exposure to airborne black carbon, which is one of major airborne particulate matter components.
Methods: Lewis Rats were exposed to ambient black carbon for two hours twice daily for five days. Corneal staining score and tear lactic dehydrogenase (LDH) activity were measured to evaluate ocular surface damage and compared with normal controls. Serum immunoglobulin (Ig) G and E were assayed using enzyme-linked immunosorbent assay, and the size of cervical lymph nodes was measured. The expression of interleukin (IL)-4, 17, and interferon (IFN)-γ in the anterior segment of the eyeball and cervical lymph nodes was measured by western blot analysis. Diquafosol tetrasodium was instilled six times a day from one day before exposure to five-day exposure duration and the ocular surface damages were evaluated and compared with saline instillation.
Results: After exposure to airborne black carbon exposure, the median corneal staining scores (0.75) and LDH activity (0.63 optical density [OD]) were significantly increased compared with those of normal controls (0, 0.44, respectively) (n=6). Serum IgG and IgE levels and the size of cervical lymph nodes were significantly increased. The expression of IL-4, IL-17, IFN-γ were elevated in the anterior segment of the eyeball and cervical lymph nodes. When exposed to airborne black carbon, topical diquafosol tetrasodium significantly increased tear MUC5AC concentration (median value, 5.83 ng/ml) and decreased tear LDH activity (median value, 0.48 OD) compared with saline instillation (2.29 ng/ml, 0.59 OD) (n=4).
Conclusions: Exposure to airborne black carbon induced ocular surface and increased pro-inflammatory cytokines in the eyes and cervical lymph nodes. Topical mucin secretagogues seem to have a protective effect on the ocular surface from the exposure to airborne particulate matters.
Commercial Relationships: Jong Suk Song; **Boram Kang**, None; **Xiangzhe Lee**, None; **Youngsub Eom**, None; **Hyo Myung Kim**, None
Support: A grant from the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (Grant No: HI13C0055)

Program Number: 452 **Poster Board Number:** A0377
Presentation Time: 1:30 PM–3:15 PM
Changes in Growth Factor Content of Human Serum for Use as Eye Drops during Frozen Storage for 1 Year
Dirk de Korte[1], Jos Lorinser[1], Pieter van der Meer[1], Hans Van Der Heiden[2]. [1]R&D, Sanquin Blood Bank, Amsterdam, Netherlands; [2]MuDrop, Apeldoorn, Netherlands.
Purpose: Growth factors are thought to be among the active components in serum used for treatment of dry-eye syndrome. The purpose of this study is to demonstrate stability of growth factor content in human serum during long-time storage at -18°C or <-25 to >-35°C packed in a new micro dose device for single use as eye drops. If these single use products can be stored at -18°C it will be feasible to store this product in household freezers, making the product more user-friendly for patients in need of serum eye drops.
Methods: Serum produced from 500 mL whole blood donations from non-remunerated healthy donors was quickly frozen. After frozen storage at <-25°C for 3-12 months and controlled thawing, six different sera were used to fill a large number of small (140 μl) containers, which were refrozen and stored at either -18^{0}C or <-25°C. During storage at 3 months intervals, samples were tested for several growth factors, using Magpix® Luminex Multiplex assays and compared to control samples stored at <-80°C. Growth factors tested were PDGF-AA&AB/BB, TGF-ß1/2/3, VEGF, EGF, FGF2. The study was a fact-finding study, without preset acceptance criteria.
Results: PDGF-AB/BB and TGF-ß1 were the most abundant growth factors, on average 35, resp. 40 ng/mL. Also PDGF-AA was detected at relatively high concentration in human serum, on average 11 ng/mL. TGF-ß2, EGF and VEGF were detected at relatively low values, resp. 3 ng/mL, 0.5 ng/mL and 0.3 ng/mL. Average levels of FGF2 and TGF-ß3 were close to detection limit (< 0.2 ng/mL). The controls stored at <-80°C showed for all growth factors close to 100 % of the initial values in fresh human serum samples at T=0. For serum stored at <-25°C for up to 12 months, less than 10% decrease was found for all tested growth factors. For serum stored at -18°C this 10 % decrease was reached after 6 months, after 12 months the decrease was 10-20%.

Conclusions: Human serum eye drops can be stored in the new micro dose device at -18^{0}C (household 3-star freezers) or <-25°C (professional freezers) for at least one year after preparation without large decreases in growth factor content. It is yet unknown if the tested components add to the *in vivo* effectiveness of serum eye drops and what the minimal concentration is to ensure *in vivo* effectiveness. Further stability testing in combination with *in vitro* and *in vivo* application is required to extend the shelf-life beyond 1 year.
Commercial Relationships: Dirk de Korte, Sanquin Blood Bank (E), Sanquin Blood Bank (F), Sanquin Blood Bank (S); **Jos Lorinser**, Sanquin Blood Bank (E), Sanquin Blood Bank (F); **Pieter van der Meer**, Sanquin Blood Bank (E), Sanquin Blood Bank (F); **Hans Van Der Heiden**, MuDrop (P), MuDrop (I)

Program Number: 453 **Poster Board Number:** A0378
Presentation Time: 1:30 PM–3:15 PM
Simultaneous wavefront and corneal topography measurement for tear film diagnostics
Daniel R. Neal[1], Jason Hoy[1], kavita P. Dhamdhere[2], Sanjeev Kasthurirangan[2], Wei Xiong[1], Thomas D. Raymond[1].
[1]Research and Development, AMO WaveFront Sciences, LLC, Albuquerque, NM; [2]Clinical Research, Abbott Medical Optics, Milpitas, CA.
Purpose: Dry Eye is a condition that affects the tear film and thus quality of vision. Tear Film Break Up Time (TBUT) measured subjectively by slit lamp is the primary clinical metric to diagnose and monitor Dry Eye Disease. It would be useful to have additional, objective metrics to gauge tear film quality. The tear film quality can be evaluated using corneal topography, aberration, lipid layer, and osmolarity. The purpose of this study is to investigate tear film dynamics using simultaneous measurements of ocular wavefront aberrations and corneal topography.
Methods: A custom laboratory instrument was developed that incorporated both Full Gradient Corneal Topography (FGCT) and wavefront aberrometry (WF). Electronics were designed to control the illumination and camera timing through rapid multiplexing. Sequences of data were recorded with WF and CT measurements at ~14 Hz rate for 40 seconds. FGCT and WF measurements were acquired in a sequence, with subjects instructed to blink and then hold their eyes open for 10-35 seconds.
Results: In ~40 seconds, 515 frames of FGCT and WF images were acquired on each subject. An example of the FGCT and WF images from near the beginning of a sequence is shown in Figure 1a, compared to the image (Figure 1b) from late in the sequence. Both WF and FGCT images have significant regions where the tear film has degraded the image spot quality after 12.6 seconds. Quantitatively, the WF RMS Zernike fit error has been shown to correlate with tear film break up. Figure 2 plots an example of this quantity as a function of time for one blink interval for the pupil area.
Conclusions: Tear film irregularity can effectively be measured using this process. A significant degradation in tear film quality was evident in both the FGCT and WF raw data. The WF Fit Error increases rapidly before a blink. Both WFFE and CTFE metrics increased as a function of time following a blink and correlate with the patient's subjective description. These metrics are potential complements to subjective measures once normative ranges have been established.

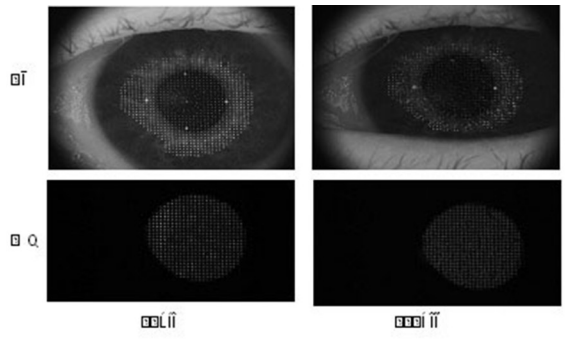

Figure 1 – FGCT and WF images at different times. T=0 corresponds to a time shortly after a blink, while T=6.7 was a image shortly before the next blink.

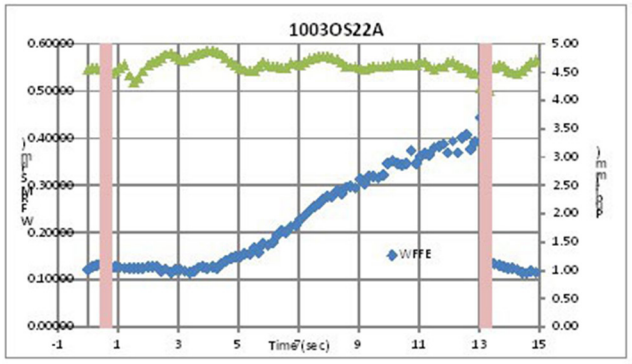

Figure 2 Time sequence for wavefront fit error.

Commercial Relationships: Daniel R. Neal, Abbott Medical Optics (P), Abbott Medical Optics (E); **Jason Hoy**, Abbott Medical Optics (E); **kavita P. Dhamdhere**, Abbott Medical Optics (E); **Sanjeev Kasthurirangan**, Abbott Medical Optics (E); **Wei Xiong**, Abbott Medical Optics (E); **Thomas D. Raymond**, Abbott Medical Optics (E)

Program Number: 454 **Poster Board Number:** A0379
Presentation Time: 1:30 PM–3:15 PM
Polymer size and other physical properties vary among hyaluronic acid-based lubricant eye drops
Peter A. Simmons[3], Pasquale Aragona[2], Hongpeng Wang[1], Tao Wang[1]. [1]Pharmaceutical Research, Allergan plc, Irvine, CA; [2]Ophthalmology, University of Messina, Messina, Italy; [3]Clinical Development, Allergan plc, Irvine, CA.
Purpose: Hyaluronic acid (HA) solutions are often used for dry eye. HA polymer size may have a large range, with higher molecular weight (MW) considered more desirable for its ability to build viscosity at lower concentration, with decreased likelihood of inflammatory reactions. Some formulations combine HA with other polymeric ingredients, which may affect the observed molecular weight, other physical properties, and clinical performance. This study assessed the MW of the HA in commercial HA formulations, along with rheological performance as a predictor of clinical efficacy, and the total osmolality and sodium content.
Methods: MW and polydispersion index (PDI) of 18 commercial eye drops were measured using a light-scattering method on a Agilent Technologies 1200 series HPLC with a Dawn Heleos-II MALS detector and an Optilab rex RI detector (both from Wyatt Technology). Rheological performance was measured on an DHR-3 rheometer (TA Instruments), with shear rate varied from 1 to 10,000 reciprocal seconds. To further characterize formulations containing polymers in addition to HA, molecular weight analysis was repeated

after hyaluronidase digestion. Total osmolality was determined with a standard osmometer, and sodium concentration ([Na+]) was determined by ICP-MS (Agilent).
Results: MW ranged from 204 to 2026 kDa, with PDI from 1.05 to 4.94. Following hyaluronidase treatment, some products decreased and others increased overall MW, indicating that HA was the larger or smaller (respectively) polymer in a mixed-polymer formulation. 5 of the 18 formulations contained relatively low (<500 kDa) MW HA, and 4 had very high (>1000 kDa) MW HA. All formulations exhibited shear-thinning, with viscosity being a function of both MW and concentration. 3 formulations had low-shear viscosity above 40 cPs, and 7 had low-shear viscosity below 10 cPs. Total osmolality ranged from 154 to 335 mOsm/kg, and Na+ content from 22 to 183 mM. Osmolality was not strictly linked to [Na+] due to contribution of alternative osmolytes.
Conclusions: Higher MW of HA allows higher low-shear viscosity at lower concentration; addition of another synergistic polymer may augment performance. Lower [Na+] may be desirable, as excess salt in the tear film is associated with up-regulation of inflammatory processes and ocular surface damage. Formulations with high-MW HA and alternative compatible osmolytes may be preferred for dry eye patients.
Commercial Relationships: Peter A. Simmons, Allergan plc (E); **Pasquale Aragona**, Allergan plc (C); **Hongpeng Wang**, Allergan plc (E); **Tao Wang**, Allergan plc (E)

Program Number: 455 **Poster Board Number:** A0380
Presentation Time: 1:30 PM–3:15 PM
Modulation of corneal and conjunctival epithelial cell mucins by glucocorticoid receptor activation: A novel mechanism for the ameliorative effect of corticosteroids
Jonathan Taniguchi[1], Marjan Farid[2], Sumit Garg[2], Ajay Sharma[1]. [1]Chapman University School of Pharmacy, Irvine, CA; [2]Gavin Herbert Eye Institute, University of California, Irvine, CA.
Purpose: Ophthalmic glucocorticoids are often used to treat ocular surface inflammation and dry eye in graft versus host disease. A decrease in ocular surface inflammatory cells and cytokine levels has been shown to contribute to the ameliorative effect of glucocorticoids. Mucins are vital for keeping the ocular surface moist and lubricated. The present study investigates the effect of glucocorticoid receptor activation on modulation of ocular surface mucins.
Methods: Human conjunctival and corneal epithelial cells were used. Conjunctival cells were grown in serum-free low calcium F12/DMEM medium then switched to serum-containing keratinocyte medium for stratification. Corneal epithelial cells were grown on transwell membrane inserts, in growth factor-supplemented keratinocyte medium. The dose and time-dependent effect of glucocorticoid receptor activation on mucin gene expression was tested by exposing the conjunctival and corneal cells to 25, 50 100 nM of fluorometholone, a glucocorticoid receptor agonist. To test whether the fluorometholone-mediated modulation of mucin expression was glucocorticoid receptor mediated, the cells were exposed to fluorometholone alone or with mifepristone (10μM), a glucocorticoid receptor antagonist. The cells were harvested at 12 and 24 hours of fluorometholone+/-mifepristone exposure. The mRNA isolation, cDNA preparation and protein extraction was performed. The mucins 1,4,5AC,16&19 gene expression and protein quantification was done using real time PCR and ELISA respectively. Statistical analysis was performed by One/Two way ANOVA and tuckey's or Bonferonni's tests.
Results: Fluorometholone caused a dose-dependent increase in the expression of ocular mucins in the conjunctival and corneal epithelial cells, and the results were statistically significant after 24 hours exposure at 50 and 100 nM dose ($p<0.01$). Mifepristone significantly ($p<0.01$) antagonized flurometholone-mediated increase in ocular mucins suggesting that increase in ocular mucins was mediated by activation of glucocorticoid receptors.
Conclusions: Glucocorticoid receptor activation increases the expression of ocular surface mucins. The observed increase in mucins may be a novel mechanism underlying the therapeutic benefits of glucocorticoids in ocular surface inflammatory diseases besides the well-documented anti-inflammatory effect.
Commercial Relationships: Jonathan Taniguchi, None; **Marjan Farid**, None; **Sumit Garg**, None; **Ajay Sharma**, None

Program Number: 456 **Poster Board Number:** A0381
Presentation Time: 1:30 PM–3:15 PM
Therapeutic efficacy of nanocomplex of poly(ethylene glycol) and catechin in a mouse model of experimental dry eye
Hyesook Lee[1], Whuisu Shim[2], Chae Eun Kim[3], So Yeon Choi[1], Haeshin Lee[2], JaeWook Yang[3, 1]. [1]T2B center for ocular diseases, Inje university Busan Paik hospital, Busan, Korea (the Republic of); [2]Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea (the Republic of); [3]Department of Ophthalmology, Inje University College of Medicine, Busan, Korea (the Republic of).
Purpose: We investigated the effect of the nanocomplex of poly(ethylene glycol) (PEG) and catechin for the treatment of dry eye disease in a mouse model.
Methods: Dry eye was experimentally induced in NOD.B10.*H2*^{b} mice through subcutaneous injections of scopolamine and exposure to an air draft for 10 days. Ten days later, the mice were treated with normal saline (n = 8), 1% catechin (n = 8), 1% PEG (n = 8), and 1% catechin/PEG nanocomplex solution mixture containing catechin and PEG at weight ratios of 1:1 (CP1, n = 8), 1:5 (CP5, n = 8), and 1:10 (CP10, n = 8). All treatments were instilled five times per day for 10 days. We estimated the effect of PEG/catechin nanocomplexes on inflammation, on tear production, epithelium stabilization, and goblet cells density.
Results: The desiccation stress significantly decreased tear production and increased the corneal irregularity score. Furthermore, the desiccation stress markedly increased the detached epithelial cells and decreased the numbers of goblet cells. In addition, the expression of pro-inflammatory-related factors was markedly induced by desiccation stress in the lacrimal glands. However, PEG/catechin nanocomplex effectively induced an increase in tear production, stabilization of corneal epithelium, and increase in conjunctival goblet cells and anti-inflammatory improvements in a PEG dose-dependent manner.
Conclusions: In this study, we found that PEG may increase bioavailability of catechin. Therefore, PEG/catechin nanocomplex can be used as new biomedical material to treat dry eye disease by stabilizing the tear film and inhibiting inflammation.
Commercial Relationships: Hyesook Lee, None; **Whuisu Shim**, None; **Chae Eun Kim**, None; **So Yeon Choi**, None; **Haeshin Lee**, None; **JaeWook Yang**, None
Support: This study was supported by a grant from the Korea Healthcare Technology R&D Project, Ministry of Health and Welfare Affairs, Republic of Korea (grant #: HI15C1142)

Program Number: 457 **Poster Board Number:** A0382
Presentation Time: 1:30 PM–3:15 PM
Calcitriol Inhibits Dry Eye Related Ocular Surface Inflammation *in vivo* and *in vitro*
Jing Zhang[1, 2], Yiqin Dai[1, 2], Dan Wu[1], JianJiang Xu[1]. [1]Ophthalmology, Eye & ENT Hospital of Fudan University, Shanghai, China; [2]Research Center, Eye & ENT Hospital of Fudan University, Shanghai, China.
Purpose: Calcitriol is the biologically active form of vitamin D_3. Many studies have shown the presence of calcitriol in the eye and its role in modulating inflammatory responses. However, the effect of calcitriol in dry eye remains unclear. This study aims to investigate the protective effects and possible mechanisms of calcitriol on dry eye related ocular surface inflammation *in vivo* and *in vitro*.
Methods: A solution of 0.2% benzalkonium chloride (BAC) was administered daily to rat eyes for 14 days, twice daily, to induce dry eye. Then rats were treated topically with (10^{-6}M per eye) or without calcitriol following BAC treatment and continuously throughout the study. The tear secretion volume, tear break-up time, and fluorescein score were measured Day 0, 5, and 10. Global specimens were collected on Day 10. Corneal damage was evaluated with HE staining. *In vitro*, human corneal epithelial cells were cultured in normal or 450 mOsm/L hyperosmolar medium with various concentrations of calcitriol. Levels of inflammatory cytokines and chemokines were measured by RT-PCR or ELISA. Level of phosphorylated IκB was detected using Western blotting.
Results: Calcitriol treatment resulted in significantly increased tear volume, prolonged BUT, and decreased fluorescein score. Histologically, corneal epithelial cell injury and inflammation were milder in rats treated with calcitriol (Figure 1). *In vitro* studies also showed that calcitriol significantly inhibited inflammatory responses triggered by hyperosmolar stress. Calcitriol markedly reduced the expression of *MIP1A* and *MIP1B*, IL-6 and IL-8 production, and NF-κB activity (Figure 2).
Conclusions: Calcitriol could inhibit dry eye related ocular surface inflammation *in vivo* and *in vitro*. The results indicated that calcitriol could be a potential therapeutic agent in the clinical treatment of dry eye.

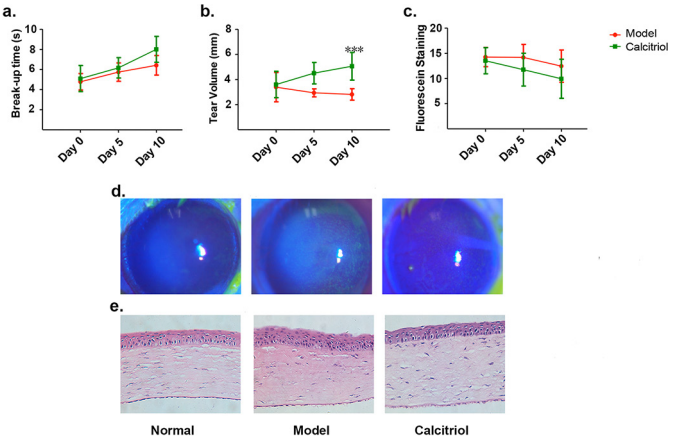

Figure 1. Calcitriol ameliorated dry eye severity.

Figure 1. Calcitriol ameliorated dry eye severity. (a) Tear break-up time; (b)Tear volume; (c) The fluorescein staining score; Representative images of corneal epithelium integrity including fluorescein staining (d) and HE staining (e).

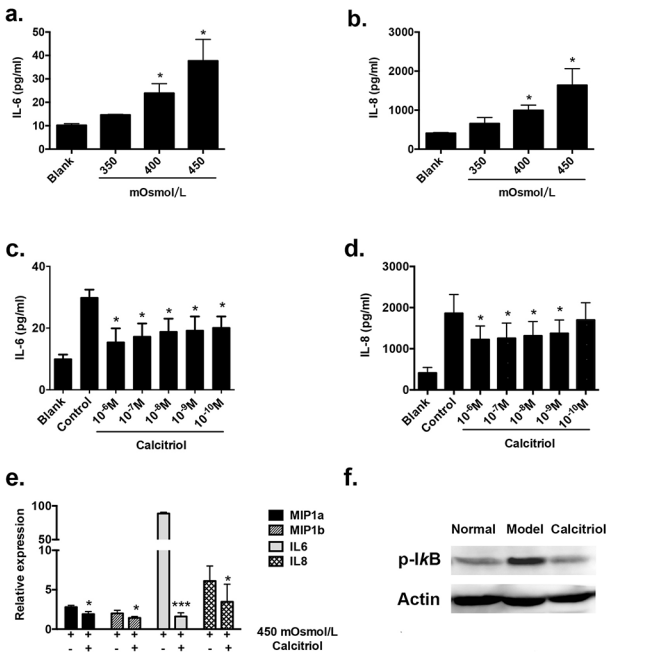

Figure 2. Calcitriol inhibited hyperosmolar stress induced inflammation *in vitro*.

Figure 2. Calcitriol inhibited hyperosmolar stress induced inflammation in vitro. IL-6 (a) and IL-8 (b) production by hHCECs after 24h exposure to hyperosmotic medium. Effects of calcitriol in inhibiting IL-6 (c), IL-8 (d) production, chemokine expression (e), and NF-κB activity (f).
Commercial Relationships: Jing Zhang, None; **Yiqin Dai**, None; **Dan Wu**, None; **JianJiang Xu**, None
Support: National Natural Science Foundation of China (81670820)

Program Number: 458 **Poster Board Number:** A0383
Presentation Time: 1:30 PM–3:15 PM
Efficacy of SYL1001 in different animal models of Dry Eye Disease
Ana Isabel Jimenez[1], Covadonga Pañeda[1], Tamara Martinez[1], Amor Guerra[1], Nuno Fonseca[1], Susana Monteiro[1], Cristian Salvador[1], Jesus Merayo-Lloves[2], Ignacio Alcalde[2], Veronica Ruz[3], Victoria Gonzalez[3]. [1]R & D, Sylentis, Tres Cantos, Madrid, Spain; [2]Instituto Universitario Fernández-Vega, Oviedo, Spain; [3]Sylentis, Madrid, Spain.
Purpose: To assess the potential role of SYL1001 in several animal models of Dry Eye Disease and to extrapolate possible uses in man.
Methods: SYL1001 is a compound based on RNA interference technology under advanced clinical development for the treatment of Dry Eye Disease. SYL1001 targets Transient Receptor Protein Vanilloid type 1 (TRPV1), a cation channel present in the neuronal terminals that innervate the cornea. Down regulation of this channel has shown to reduce eye discomfort in patients suffering from Dry Eye Disease (measured using the VAS scale) as well as conjunctival hyperemia staining. The primary objective of this set of studies was to assess different outcome measures in animal models of Dry Eye Disease and to compare the efficacy of SYL1001 to that of other treatments currently available in the clinic. For this purpose we used a mouse model in which dry eye symptoms were induced in C57BL/6N mice by exposing them to a controlled environment (relative humidity <25%, airflow 15L/min, temperature 20-22°C) and systemic scopolamine administration (0.5 mg/72h) for 7 days.

To further elucidate the possible actions of SYL1001 in dry eye disease, a mouse model of dry eye induced by corneal ablation was used. In this model the innervation of the central cornea is completely severed, causing a reduction in tear production.
Results: RNA interference works in a sequence dependent manner, reducing specifically the expression of the gene against which the compound is designed. Prior to conducting experiments in murine models we analyzed if SYL1001 was active in mouse cells by transfecting the compound into C2C12 cell line. The compound was shown to reduce TRPV1 mRNA levels in these cells with an efficacy comparable to the one observed in human cells. Treatment with SYL1001 dose-dependently increased the number of MUC5A+ positive cells in the cornea of a mouse model of scopolamine-induced dry eye disease; this effect was equivalent to that of cyclosporine. In the corneal-ablation model, treatment with SYL1001 improved the rate of corneal wound healing and haze scoring. In addition, SYL1001 increased tear production if the first days post-ablation.
Conclusions: SYL1001, a RNA compound under development for the treatment of signs and symptoms of dry eye disease has shown to improve several outcomes in different models of dry eye disease. This further supports the development of the compound for several forms of dry eye disease.
Commercial Relationships: Ana Isabel Jimenez, Sylentis (E); **Covadonga Pañeda**, Sylentis (E); **Tamara Martinez**, Sylentis (E); **Amor Guerra**, Sylentis (E); **Nuno Fonseca**, Sylentis (E); **Susana Monteiro**, Sylentis (E); **Cristian Salvador**, Sylentis (E); **Jesus Merayo-Lloves**, Sylentis (C); **Ignacio Alcalde**, None; **Veronica Ruz**, Sylentis (E); **Victoria Gonzalez**, Sylentis (E)
Support: Spanish Goverment Grant, SURFEYE & SEKEYE

Program Number: 459 **Poster Board Number:** A0384
Presentation Time: 1:30 PM–3:15 PM
Caspase-8 promotes the activation of NLRP3 inflammasome and inhibits the production of NLRP6 in dry eye disease
Yonghao Li, Wei Chi. Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China.
Purpose: Dry eye disease (DED) is the most common ocular surface disease, average one out of every five persons suffered by this disease in the worldwide. Accumulating evidence demonstrates that immunoinflammatory mechanisms play pivotal roles in the pathogenesis of DED. However, the potential roles and mechanism of innate immune are largely unknown.
Methods: In the present study, we used an experimental murine dry eye model and in vitro human limbal epithelial cell (HLEC) dry eye model induced by hyperosmotic stress to investigate the underlying mechanisms of innate immune in DED.
Results: We found that caspase-8 activated by ROS play a key role in the pathogenesis of DED by forming a caspase-8-ASC inflammasome, promoting the activation of NLRP3 inflammasome and downregulating NLRP6 inflammasome via NF-κB pathway. The activation of NLRP3 inflammasome can also suppress the production of NLRP6 and promote the processing of IL-1β and IL-18, actively involving in the innate immune responses of DED.
Conclusions: These findings demonstrate collectively a critical role of caspase-8-inducing NLRP3 inflammasome activation in processing IL-1β and IL-18 maturation and decreasing the expression of NLRP6 in DED. These results provide new insight into the pathogenesis of DED and point to a treatment strategy.
Commercial Relationships: Yonghao Li, None; **Wei Chi**, None

Program Number: 460 **Poster Board Number:** A0385
Presentation Time: 1:30 PM–3:15 PM
Rabbit Safety of Topical PPL-003: A Cell-Penetrating Peptide Inhibitor of NFkB for Dry Eye Disease
Bruce H. Littman[1, 2], Jeffrey A. Jamison[3], Ricardo Ochoa[4]. [1]Portage Pharmaceuticals Ltd., Stonington, CT; [2]Translational Medicine Associates, LLC, Savannah, GA; [3]Ophthy-DS, Inc., Portage, MI; [4]Pre-Clinical Safety, Inc., Niantic, CT.
Purpose: Topical PPL-003 administration for 28 days was evaluated for safety and toleration in NZW rabbits.
Methods: Twelve rabbits were assigned to 4 topical treatment groups and ocular safety was assessed over 28 days (see table). Animals were euthanized on Day 28; eyes were harvested and fixed. Representative H&E stained sections were examined microscopically. PPL-003 plasma levels and serum IgG antibodies to PPL-003 were determined by ELISA.
Results: Vehicle and PPL-003 treated eyes were generally normal. Conjunctival erythema was noted sporadically with no relation to dose or timing. No cataracts, vitreal or retinal changes or IOP increases occurred. Some inflammatory cells were visible in the anterior chamber with no relation to dose and also occurred in the vehicle group. On Day 23 auto/hyper fluorescence was noted in the periphery of one eye in one animal at the 5.0 mg/ml dose and in both eyes of one animal at the 2.5 mg/ml dose. OCT examination revealed disorganization of the photoreceptor layer or possibly a small retinal detachment. The fundus and vasculature of the remaining eyes appeared normal. Histologically the ciliary body, iris, lens, vitreous, retina and choroid tissues were normal in all rabbits. Dose-related minimal (Grade 1) or mild (Grade 2) mononuclear cell infiltration of conjunctival tissue without erosion of the epithelium was noted in some rabbits. Eight of 9 rabbits that received PPL-003 had anti-PPL-003 IgG antibodies by Day 21. PPL-003 was not detected (<2.6 ng/ml or 3.0 X 10^{-13} M) in any plasma samples.
Conclusions: Topical TID PPL-003 at concentrations of 0.5 – 5.0 mg/ml were well tolerated with no significant in-life or histopathological findings other than mild dose-related conjunctival inflammation. Imaging abnormalities in 3 eyes were possibly stress-related since retinal histology was normal. No systemic PPL-003 exposure was found. PPL-003 with its human derived CPP sequence was immunogenic in rabbits.

Group	N	Topical Treatment	PPL-003 Concentration	Clinical Obs. and IOP	Fluorescein and ICG Angiography with OCT	ERG	Plasma and Serum for PK and Immunogenicity
1	3	50 ul OU TID Day 0-28	5 mg/ml	Pre-treatment and prior to dosing on Days 7, 14, 21 & 28	Days 11 & 23	Pre-treatment Days 9/10 & 24/25	Days 0 & 21 2 hours after dosing
2	3		2.5 mg/ml				
3	3		0.5 mg/ml				
4	3		Vehicle				

Study Design.
Commercial Relationships: Bruce H. Littman, Portage Pharmaceuticals Ltd. (C), Portage Pharmaceuticals Ltd. (I), Translational Medicine Associates, LLC (E); **Jeffrey A. Jamison**, Portage Pharmaceuticals, Ltd. (C); **Ricardo Ochoa**, Portage Pharmaceuticals Ltd. (C)

Program Number: 461 **Poster Board Number:** A0386
Presentation Time: 1:30 PM–3:15 PM
Conjunctival goblet cells produce bioactive retinoic acid that modulates dendritic cell cytokine signaling
Yangyan Xiao[1, 2], Cintia S. De Paiva[1], Terry G. Coursey[1], Fang Bian[1], De-Quan Li[1], Stephen C. Pflugfelder[1]. [1]OPHTHALMOLOGY, BAYLOR COLLEGE OF MEDICINE, Houston, TX; [2]Ophthalmology, Second Xiangya Hospital, Changsha, China.
Purpose: Goblet cells (GCs) are in close proximity to dendritic cells (DCs) in the conjunctival epithelium and stroma. We hypothesized that GCs produce retinoic acid (RA) that modulates cytokine signaling in DCs.
Methods: Primary murine conjunctival and corneal epithelia grown from explants were cultured for 7 days or added diethylaminobenzaldehyde (DEAB) on day 5, then switched to IMDM for 4 days when conditioned media from conjunctiva (CjCM) and cornea (KCM) were collected. Bone marrow-derived dendritic cells (BMDCs) were cultured for 6 days in IMDM, then conditioned for 2 days with CjCM or exogenous RA. Quantitative real-time PCR (RT-PCR) and ALDEFLUOR activity assay were performed to compare ALDH1 expression and ALDH (aldehyde dehydrogenase) activity. RA concentration in supernatants from cultures with or without retinol palmitate(RP)supplementation was detected with F9-RARE-lacZ reporter cells. Expression of the RA inducible gene SOCS3, IFN-a1 and Th1 associated genes was measured by RT-PCR in control BMDCs and those treated with CjCM or exogenous RA with or without adding 1mM mm11253 (RARγ antagonist) or 1μg/μl lipopolysaccharide (LPS).
Results: Conjunctival GCs had greater production of RA than cornea (Fig.1a), supported by higher levels of ALDH1 and had higher ALDEFLUOR activity in GCs than cornea. RA in CjCM increased with RP supplementation.
CjCM increased SOCS3 and down regulated IFN-γ and IFN-a1 in DCs, similar to exogenous RA (Fig.1b). DCs in CjCM showed a tolerogenic phenotype with reduced expression of co-stimulatory molecules CD86 and increased anti-inflammatory (IL-10) cytokines. Furthermore, CjCM suppressed LPS stimulated MHC class II and pro-inflammatory cytokine (IL-1ß, IL-12, IL-23) expression. However, DEAB (the ALDH inhibitor) added to GC culture reduced the effect. DEAB also inhibited an increase in ALDH activity with CjCM in DCs. Addition of the RAR-γ antagonist decreased expression of SOCS3 (0.71±0.11 $P<0.05$; 0.47±0.01 $P<0.01$) and increase IL-1ß (2.17; 2.51±0.19) in response to CjCM or RA, respectively.
Conclusions: Our study demonstrates that GCs can convert tear retinol to RA, which increases SOCS3 expression and inhibits Th1 inducing cytokines in DCs. GCs appear to play an important role in regulating DC maturation and maintaining ocular surface immune tolerance.

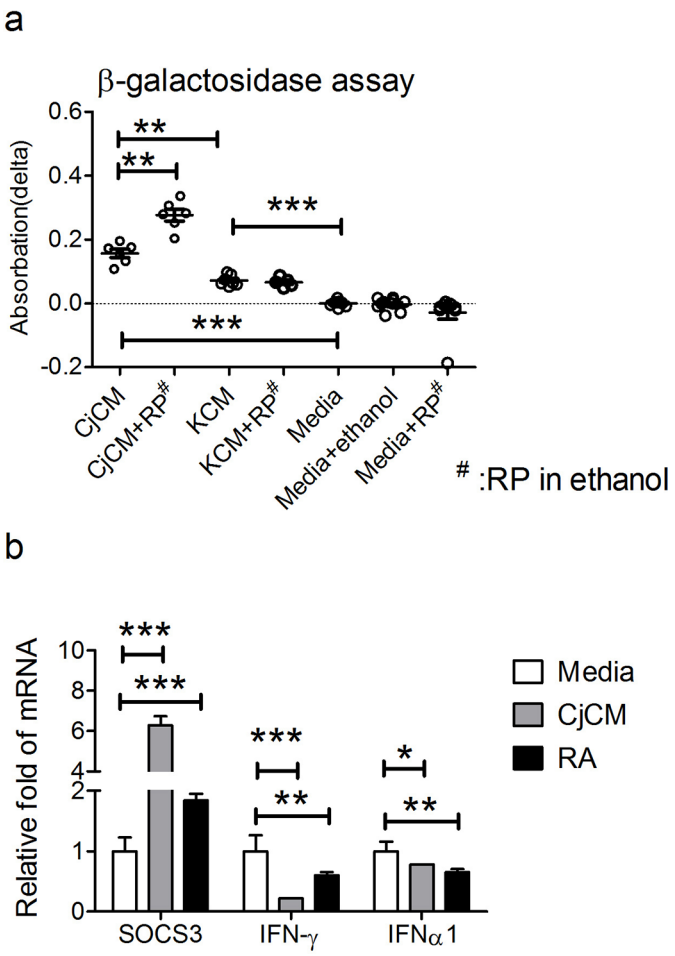

Figure 1
Commercial Relationships: Yangyan Xiao, None; **Cintia S. De Paiva**, None; **Terry G. Coursey**, None; **Fang Bian**, None; **De-Quan Li**, None; **Stephen C. Pflugfelder**, None
Support: by NEI grant EY-11915 (SCP), NEI/NIH Core Grant EY-002520, Biology of Inflammation Center Baylor College of Medicine, Research to Prevent Blindness, The Oshman Foundation, William Stamps Farish Fund and The Hamill Foundation, CSC Scholarship

Program Number: 462 **Poster Board Number:** A0387
Presentation Time: 1:30 PM–3:15 PM
Intravenously Injected Autologous, Ex Vivo-Activated Lymphocytes Adoptively Transfer Dry Eye Disease to Rabbits
Austin K. Mircheff[1], Yanru Wang[1], Houman Hemmati[2], Luis Rodriguez-Borlado[2]. [1]Dept of Physiology & Biophysics, Univ of Southern California, Los Angeles, CA; [2]Capricor Therapeutics, Inc, Beverly Hills, CA.
Purpose: Dry eye disease is a manifestation of chronic, immune mediated inflammatory processes in the ocular surface tissues and lacrimal glands (LG). Biologically appropriate animal models are needed. Of theoretical and practical importance, immunocompetent rabbits are susceptible to adoptively-transferred DED, while immunocompetent rodents are not. Accordingly, we have designed a new, efficient protocol for adoptively transferring immune-mediated ocular surface inflammation to rabbits.
Methods: Microparticles (M_p), which include exosomes that LG acinar cell secrete constitutively, are naturally microencapsulated

samples of autoantigens from both the cytosol and the cells' membrane-bounded compartments. M_p were isolated from supernatant primary culture medium and used to prime dendritic cells (mDC) that had been matured from bone marrow monocytes and stimulated with LPS. Peripheral blood lymphocytes (PBL) were isolated from study animals (n=6); activated in ex vivo mixed cell reactions with M_p-primed mDC; then reintroduced autologously via marginal ear veins. Ocular surface status was evaluated by rose Bengal (RB) staining and Schirmer I tear tests (STT-I) at baseline and then biweekly for 12 wk after PBL injection.

Results: One eye with high baseline RB score (1.5) and low STT-I score (6.0 mm/min) was excluded as spontaneously diseased. Ocular surface inflammation developed over bi-phasic time-courses in the remaining 11 eyes. RB scores increased to 0.8 ± 0.1 above baseline value of 0.8 ± 0.1) at wk 2, subsided at wk 2, and recrudesced at wk 8, suggesting a biphasic phenomenon. By wk 12, RB scores remained elevated 1.0 ± 0.1 above baseline in 5 eyes and 0.5 ± 0.1 above baseline in 5 eyes, but returned to baseline in 1 eye. STT-I scores decreased 3.4 ± 0.4 mm below baseline value of 9.0 ± 0.5 in 8 eyes, remained unchanged in 2 eyes, and increased 1.0 mm above baseline in 1 eye. By wk 12, STT-I scores remained decreased 2.1 ± mm in 7 eyes, unchanged in 1 eye, and increased 2.0 mm above baseline in 1 eye. The scores remitted together in only 1 eye. Preliminary H&E demonstrated extensive fatty infiltratrion in 1 LG and both inter-acinar infiltrates and periductal infiltrates of varying sizes and frequences in the remaining LG.

Conclusions: This may be an appropriate model for pathophysiology and treatment of mild-to-moderate DED.

Commercial Relationships: Austin K. Mircheff, None; **Yanru Wang**, None; **Houman Hemmati**, Capricor Therapeutics, Inc (E); **Luis Rodriguez-Borlado**, Capricor Therapeutics, Inc. (E)

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Presentation Time: 1:30 PM–3:15 PM

Lack of Goblet Cells in SPDEF KO Mice Increases Retention of IL-12 Producing Dendritic Cells in Conjunctiva

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Purpose: Goblet cell (GC) mucins have been found to condition tolerogenic properties in intestinal dendritic cells (DCs). Aqueous tear deficiency is associated with GC loss and greater IFN-γ expression in the conjunctiva (Cj). We hypothesize that loss of Cj GCs is associated with greater dendritic cell T helper (Th)1 polarization in the SPDEF knock-out (KO) strain that lacks GCs.

Methods: Six to nine week-old female C57BL/6 wild type (WT) and SPDEF KO mice were used. $CD11c^+$ and $CD11b^+$ DCs were visualized in whole mount Cj by laser scanning confocal microscope using the Z-stack option. IL-12 expression in DC was quantified by flow cytometry and $IL\text{-}12^+$ cells were also detected by immunostaining in Cj cryosections. Mixed lymphocyte reactions to evaluate T helper (Th) cell polarization and proliferation were performed with $OVA_{323\text{-}339}$ peptide pulsed cervical lymph node (CLN) cell suspensions and OT II $CD4^+$ T cells. Th cytokine expression (IFN-γ and IL-17) was measured by flow cytometry and immunobead assay. The effects of WT conjunctival conditioned medium (CJCM) on lipopolysaccharide (LPS) -stimulated IL-12 expression in the Cj was evaluated after topical application in SPDEF KO mice.

Results: SPDEF KO had greater frequency of $CD11b^+CD11c^-$ ($p=0.01$) and $CD11b^-CD11c^+$ conjunctival DCs and $CD11b^-CD11c^+$ DCs were more superficially located in WT mice. Immunohistochemistry demonstrated that SPDEF KO mice had a greater number of $IL\text{-}12^+$ cells in Cj stroma than WT group. A greater percentage of $CD11b^+CD11c^-$ and $CD11b^+CD11c^+$ cells ($p \leq 0.03$) with higher IL-12 median fluorescent intensity (MFI) (both $p \leq 0.03$) in the Cj compared to WT control was demonstrated by flow cytometry. Th cells primed by SPDEF KO CLN DCs showed a skewing towards Th1 and generated a lower frequency of $CD4^+Foxp3^+$ cells. Significantly increased IFN-g was measured in the supernatant of these cells ($p=0.03$). Following topical LPS stimulation, SPDEF KO mice had higher IL-12 expression in $CD11b^+CD11c^+$ Cj DCs than the control group ($p=0.02$) and reconstitution with topically applied WT CJCM reduced stimulated IL-12 production in $CD11b^+CD11c^+$ cells compared to vehicle control ($p=0.04$).

Conclusions: Our results support our hypothesis that CJ GCs have a tolerogenic effects on DCs by suppressing IL-12 production and Th1 polarization.

Commercial Relationships: Byung Yi Ko, None; **Yangyan Xiao**, None; **Fang Bian**, None; **Jeffrey Whitsett**, None; **Hans Clever**, None; **Flavia L. Barbosa**, None; **Cintia S. De Paiva**, None; **Stephen C. Pflugfelder**, None

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Narrow Spectrum Kinase Inhibitor (NSKI) Targets Are Up-regulated in Conjunctival Cells from Dry Eye Patients Compared to Healthy Controls

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Purpose: Dry Eye Syndrome (DES) is a debilitating disease affecting up to 30% of the over-50s population. Although disease pathogenesis is multifactorial, inflammatory mechanisms are a key driver of DES symptoms (burning, irritation, redness) in most patients with moderate-to-severe disease. Treatment options for these patients are limited, with topical cyclosporine and Lifitegrast being the only FDA-approved therapeutics. Here we investigate the potential of NSKIs as a treatment for DES and demonstrate that the kinases targeted are up-regulated in conjunctival cells of patients compared to healthy controls (HCs).

Methods: Inhibitory kinase activity of the NSKI TOP1362 was assessed in competition binding assays, with determination of dissociation constants (K_d, KinomeScan™) for P-38 alpha (P38-α) and spleen tyrosine kinase (Syk), and inhibition of substrate phosphorylation (ZLYTE™) for Src activity. Human conjunctival cells from DES patients (n=9) and HCs (n=8) were harvested by impression cytology using the Eyeprim™ device. Following morphological assessment, quantitative polymerase chain reaction (qPCR) was performed to quantify differences in expression of P38-α, Src kinase and Syk. In addition, the effects of TOP1362

(0.01 – 1 μg/ml) on p38-α expression in Chang cells challenged with 100mM NaCl for 6 hours, was assessed.
Results: TOP1362 is a potent inhibitor of P38-α and Syk kinases with Kd values of 26 and 18nM, respectively and an IC_{50} of 14 nM in the Src kinase activity assay. Using the Eyeprim device, the average yield of human conjunctival cells was 1.1 x10^5cells/mm^2. qPCR analysis demonstrated significant up-regulation of Syk ($P<0.0001$), P38-a ($P<0.001$) and Src ($P<0.001$) in conjunctival cells isolated from DES patients compared to HCs. Hyperosmolar challenge of Chang cells with NaCl led to a statistically significant increase in P38-α expression. TOP1362 dose-dependently inhibited this up-regulation.
Conclusions: TOP1362 potently inhibits P38-α, Src and Syk; key kinases involved in inflammatory signalling cascades. Here, we demonstrate that these kinases are significantly up-regulated in conjunctival cells of DES patients compared to HCs, demonstrating the potential utility of NSKIs as a treatment option for this debilitating disease.
Commercial Relationships: Suzanne Hagan; **Boatemaa Omotayo**, Topivert Pharma Ltd (F); **Martyn R. Foster**, Topivert Pharma Ltd (E); **Yemisi Solanke**, Topivert Pharma Ltd (E); **Sameer Sirohi**, Topivert Pharma Ltd (E); **Katherine Oliver**, Topivert Pharma Ltd (F); **Michael J. Doughty**, Topivert Pharma Ltd (F); **Steve Webber**, Topivert Pharma Ltd (E); **Claire A. Walshe**, Topivert Pharma Ltd (E)
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INFRARED THERMOGRAPHY OF THE OCULAR SURFACE OF TEAR-DEFICIENT EYES TREATED WITH PERFLUOROHEXYLOCTANE
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Purpose: To study ocular surface temperature before and after the instillation of perfluorohexyloctane (PFHO) in tear-deficient guinea pigs.
Methods: Four young guinea pigs of both sexes were studied 4 weeks after surgical removal of the main lachrymal gland to induce tear-deficiency (DE). Infrared video images were obtained (IR thermal camera InfRec R300SR, Nippon Avionics) and analyzed using dedicated software. Ocular surface temperature was measured in the center of the cornea (CST) and the temporal conjunctiva (CJST), immediately after eye opening (T0) and 5 (T5) and 10 (T10) seconds afterwards. Slopes of temperature decay (T0/T5 and T0/T10) were also calculated. Temperature measurements were done before and 2 and 10 min after a single 10μl drop of PFHO. Tearing rate was also measured using phenol red threads placed in the nasal canthus for 30 s. The same measurements were done in 4 control animals in order to compare the data.
Results: Before PFHO, CST was slightly higher in DE than in control animals (Table 1). PFHO treatment evoked a transient increase of blinking and a slightly increased the tearing rate in DE and control animals. CST was significantly decreased at 2 and 10 min after PFHO treatment both in control and DE animals (Table 1). T0/T5-CST slope was faster in PFHO-treated eyes (-0.06±0.02°C/s vs -0.20±0.01°C/s, $p<0.05$). CJST was also higher in DE than in control eyes ($p<0.05$). PFHO reduced CJST ~1.5°C, to values similar to those of control eyes.
Conclusions: Perfluorohexyloctane forms a protective layer over the tear film that maintains the cornea and the conjunctiva cooler at least 10 min after its application. Reduced ocular surface temperature acts probably as a sustained stimulus for cold thermoreceptors involved in maintaining basal tearing.

Table 1	CST (°C)		
	T0	**T5**	**T10**
Tear deficient			
Before PFHO	37.1±0.2	36.8±0.2	36.8±0.2
2 min PFHO	35.6±0.2*	34.6±0.2*	34.7±0.3*
15 min PFHO	35.9±0.3*	35.3±0.3*	35.2±0.4*
Control			
Before PFHO	36.9±0.1	36.5±0.1	36.4±0.1
2 min PFHO	35.2±0.3*	34.0±0.2*	34.0±0.3*
15 min PFHO	35.2±0.2*	35.6±0.3*	34.4±0.5*
*p<0.05 Dunn's or Holm-Sidak, compared with "Before PFHO".			

Commercial Relationships: M Carmen Acosta, None; **Carolina Luna**, None; **Susana Quirce**, None; **Juana Gallar**, None
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Presentation Time: 1:30 PM–3:15 PM
Substance P Mediates Dysfunction of $NK1R^+$ Tregs in Dry Eye Disease
Anna Marmalidou[2], Yihe Chen[2], Chunyi Shao[2], Takeshi Nakao[2], Sunil Chauhan[2], Reza Dana[1]. [1]Ophthalmology, Schepens Research Institute/Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA; [2]Ophthalmology, Schepens Eye Research Institute/Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA.
Purpose: Dysfunction of regulatory T (Treg) cells has been implicated in the pathogenesis of dry eye disease (DED). Substance P (SP) is a neuropeptide involved in modulation of immune responses. Recent studies have shown the expression of SP and its receptor NK1R by immune cells. However, the role of SP in the pathogenesis of DED is mostly unknown. The aim of this study was to investigate the expression levels of SP, $NK1R^+$ Treg frequencies, and their Foxp3 expression in a mouse model of DED.
Methods: Wild-type (WT) C57BL/6 mice were exposed to desiccating stress using a low humidity contolled environment chamber for 14 days to induce DED. Mice were then housed in a standard environment with normal humidity for additional 7 days. Expression levels of SP were quantified by real-time PCR of samples harvested from draining lymph nodes, corneas, and conjunctivas of WT (n=7) and DED (n=21) mice at days 7, 14, and 21. Frequencies of $NK1R^+$ Tregs and their Foxp3 expression (mean fluorescein intensity, MFI) were assessed by flow cytometry analysis of $NK1R^+$ Tregs derived from the draining lymph nodes of WT (n=6) and DED (n=18) mice at days 7, 14, and 21.
Results: Our RT-PCR data demonstrated that expression of SP increases on day 21 in the draining lymph nodes, on days 7, 14, and 21 in the cornea, and on days 2 and 4 in the conjunctiva of mice with DED. Analysis of the draining lymph nodes of DED mice showed significantly increased frequencies of $NK1R^+$ Tregs on day 21 (WT: 27.84% ± 2; DED: 41.9% ± 4; $P < 0.05$). In addition, expression of Foxp3 (a master regulator of Treg function) was significantly suppressed in $NK1R^+$ Tregs on days 14 and 21 compared to WT mice (MFI=4027 ± 330 vs. 5363 ± 933 on day 14, and 3746 ± 473 vs. 5363 ± 933 on day 21; $P < 0.05$).
Conclusions: Our data demonstrate increased levels of SP, increased frequencies of $NK1R^+$ Tregs, and decreased Foxp3 expression by

$NK1R^+$ Tregs in mice with DED, suggesting that high levels of SP could be associated with Treg dysfunction in DED.
Commercial Relationships: Anna Marmalidou, None; **Yihe Chen**, None; **Chunyi Shao**, None; **Takeshi Nakao**, None; **Sunil Chauhan**, None; **Reza Dana**, None
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Presentation Time: 1:30 PM–3:15 PM
Mechanism in suppression of CCK pathway by rebamipide in trigeminal neurons
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Purpose: Recently, corneal hypersensitivity has been reported to participate in the pathogenesis of dry eye disease. Rebamipide ophthalmic suspension, which is widely used in the treatment of dry eye disease, is known to repair the milieu of keratoconjunctival epithelia and increase mucin production in conjunctival goblet cells. In addition to the restoration of ocular surface, we also demonstrated that rebamipide ameliorated the pain sensation of dry eye patients and that rebamipide reduced intracellular calcium concentration caused by activation of transient receptor potential channel subfamily member V1 (TRPV1), a pain receptor, through inhibition of neuropeptide cholecystokinin (CCK) pathway. In this study, we further explored the detailed pharmacological mechanism by which rebamipide inhibits CCK pathway.
Methods: After euthanasia, trigeminal ganglion (TG) cells were harvested from Wistar rats (1- to 3-week-old). We examined the expression of CCK and CCK receptors (CCK-1R and -2R) by reverse transcriptional PCR and *in situ* hybridization. The effect of CCK-1R and -2R antagonists in the cultured TG cells treated with CCK (10^{-7}M) was evaluated by intracellular calcium concentration measurement. Additionally, we quantified the binding affinity between rebamipide and CCK receptors using radioisotope-labelled ligand binding assay.
Results: We confirmed the expression of CCK, CCK-1R and -2R in rat TG cells. The intracellular calcium concentration of rat TG cells increased by CCK stimulation (41.2±10.7nM, n=32).
CCK-1R antagonist did not suppress the response (38.3±12.5nM, n=32, P=0.47). By contrast, CCK-2R antagonist suppressed the increase of intracellular calcium concentration caused by CCK stimulation (1.1±0.14nM, n=32, P<0.01). However, the binding affinity of rebamipide to CCK-1R (IC_{50}=4.70x10^{-5}M) was higher than to CCK-2R (IC_{50}>1x10^{-3}M).
Conclusions: The current data demonstrate that rebamipide preferably binds to CCK-1R; however, CCK induces the increase of intracellular calcium concentration through CCK-2R signaling in rat TG cells.
Commercial Relationships: Yoshiaki Tagawa, None; **Kousuke Noda**, None; **Ken-ichi Otsuguro**, None; **Erdal T. Ishizuka**, None; **Atsuhiro Kanda**, None; **Susumu Ishida**, None
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Program Number: 468 **Poster Board Number:** A0393
Presentation Time: 1:30 PM–3:15 PM
Topical instillations of Benzalkonium Chloride alter the extracellular activity of the ciliary nerve
Fanny Joubert[1], Laurence Bodineau[5], M Carmen Acosta[2], Juana Gallar[2], Jose A. Sahel[3, 4], Christophe Baudouin[1, 4], Stéphane MELIK PARSADANIANTZ[1], Annabelle Reaux-le Goazigo[1].
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Purpose:
Ocular surface diseases are among the most frequent ocular pathologies, with prevalence ranging between 10 and 20% of the general population. Benzalkonium chloride (BAK) as a preservative is a major cause of dry eye in patients treated over the long term like in glaucoma. Here we investigated the effect of an acute exposure of BAK to the cornea on the extracellular ciliary nerve activity using an *ex vivo* preparation of isolated mouse whole eye.
Methods:
Adult male C57BL/6 mice (8 weeks old) were used. Eye was placed in the two-compartment chamber and the extracellular spontaneous activity of the ciliary nerve was recorded. Two types of BAK application were used: *i) ex vivo*: BAK instillations were directly performed during the ciliary nerve recording and *ii) in vivo*: mice received repeated instillations of BAK and after eyes were removed and multiunit ciliary nerve activity was recorded. 0.02% BAK was used for *in vivo* and *ex vivo* experiments (15 instillations - 5 min intervals) and 0.2% BAK was used for *ex vivo* application (3 times - 15 min intervals). The mechanical threshold response was determined using von Frey filament before and after BAK exposure.
Results:
The electrophysiology method to record multiunit ciliary nerve activity in mouse preparations is accurate and reliable. Basal activity of ciliary nerve is 24.6±5.3imp/sec. We showed that *ex vivo* instillation of 0.02% BAK increased impulse activity of corneal nerve followed by a decreased activity of the ciliary nerve. Importantly, we observed a modified mechanical threshold response following BAK treatment. Similar results were obtained after instillations with 0.2% BAK. A first application of 0.2% BAK significantly increased the ciliary nerve activity (~+50%). A second application 15 min later increased the activity (but less strongly; ~+20% compared to basal activity) and after the third instillation, ciliary nerve activity clearly decreased (~-50%). Interestingly, we observed a higher basal activity of the ciliary nerve after *in vivo* instillations of 0.02% BAK compared to control eye.
Conclusions:
This work presents the methodology to record the ciliary nerve activity in mouse preparation. Our electrophysiological results provide the first evidence that corneal exposure to BAK altered the ciliary nerve fibers. These experiments constitute a first step to better understand the corneal neurotoxicity of BAK.
Commercial Relationships: Fanny Joubert, None; **Laurence Bodineau**, None; **M Carmen Acosta**, None; **Juana Gallar**, None; **Jose A. Sahel**, None; **Christophe Baudouin**, None; **Stéphane MELIK PARSADANIANTZ**, None; **Annabelle Reaux-le Goazigo**, None

Program Number: 469 **Poster Board Number:** A0394
Presentation Time: 1:30 PM–3:15 PM
Dynamic Sensitivity of Corneal TRPM8 Receptors to Menthol Instillation in Dry Eye vs Normal Subjects
Peter Corcoran[2], Michael Watson[1], George W. Ousler[1], Endri Angjeli[2], Keith J. Lane[2], Mark B. Abelson[3], David A. Hollander[3]. [1]Dry Eye, Ora, Inc, Andover, MA; [2]R&D, Ora, Inc, Andover, MA; [3]Ora, Inc, Andover, MA.
Purpose: To assess the sensitivity of corneal cold receptors to a known TRPM8 agonist, menthol, in dry eye and normals and determine whether factors, such as disease duration or age, affect responses.
Methods: Dry Eye (DED; N=33) and normal (N=15) subjects were randomly assigned to receive Rohto® Hydra (0.01% menthol) or Systane® Ultra treatments (OU) in a prospective, double-blind, cross-over study. DED subjects had documented disease and symptom response scores >2 on a 0 to 5-point scale. Normals had no history of DED and scores <2 on the same scale. Endpoints included mean cooling score (MCS: 0=not cool; 10=very cool, 10=highest possible score) evaluated at 0-minutes (m), 0.5m, 1m, 2m, 3m, 4m post-instillation; sum cooling scores (SCS: 5 time points summed, 60=highest possible score), and ocular signs (staining, TFBUT, Schirmer's test) and symptoms. Corneal sensitivity was assessed with Cochet-Bonnet esthesiometry. Subgroups of </>10 years DED-duration were assessed
Results: MCS at 0.5-4m post-menthol instillation were significantly higher in DED ($p \leq 0.03$). Corneal sensitivity scores based on Cochet bonnet mechanical sensitivity) were not different between groups. The duration of dry eye disease based on < 10-yr (N=18, 28.3 ± 2.58) versus >10 years (N=15, 20.2 ± 2.76) was directly correlated with MCS, while no statistically significant differences were found in MCS based on age. Similar tear production (Schirmer's score), corneal staining, TFBUT, and symptoms were also observed in the two patient populations with different duration of DED.
Conclusions: DED subjects had greater sensitivity to the TRPM8 agonist, menthol, than normal subjects. DED duration, and not age, was critical to cooling sensitivity. The finding that cooling scores were higher in subjects with DED for fewer than 10-years compared to more than 10-years suggests that corneal cold receptor sensitivity decreases as the duration of DED increases. These findings reveal new possibilities for future study of the role TRPM8-mediated sensory dysfunction plays in ineffective compensatory mechanisms in dry eye, and how targeting these aspects of pathophysiology might benefit patients.
Commercial Relationships: Peter Corcoran, Ora, Inc (E); **Michael Watson**, Ora, Inc (E); **George W. Ousler**, Ora, Inc (E); **Endri Angjeli**, Ora, Inc (E); **Keith J. Lane**, Ora, Inc (E); **Mark B. Abelson**, Ora, Inc (E), Ora, Inc (P); **David A. Hollander**, Ora, Inc (E)
Clinical Trial: NCT02985827

Program Number: 470 **Poster Board Number:** A0395
Presentation Time: 1:30 PM–3:15 PM
Enhanced natural tearing by electrical stimulation of the anterior ethmoid nerve
Mark Brinton[1], Andrea Kossler[2], Zara Patel[3], Jim Loudin[4], Manfred Franke[5], Chris Ta[2], Daniel V. Palanker[2, 4]. [1]Electrical Engineering, Stanford University, Stanford, CA; [2]Ophthalmology, Stanford University, Stanford, CA; [3]Otolaryngology, Stanford University, Stanford, CA; [4]Hansen Experimental Physics Laboratory, Stanford University, Stanford, CA; [5]Independent Consultant, Los Angeles, CA.
Purpose: Electro-neural stimulation enhances tear secretion to treat dry eye disease. We evaluated the effects of electrical stimulation of the anterior ethmoid nerve (using a chronically implanted neurostimulator) on secretion of the tear aqueous, lipid and protein content.
Methods: Neurostimulators were implanted beneath the nasal mucosa in New Zealand white rabbits. Stimulations (2.3-2.8mA pulses of 75-875μs in duration repeated at 30-100Hz for 3 minutes) were performed daily, for three weeks to measure changes in tear volume (Schirmer test), osmolarity (TearLab® osmometer), lipid (Oil-Red-O staining) and protein (BCA assay, mass-spectrometry).
Results: Stimulation of the anterior ethmoid nerve in the frequency range of 30-100Hz increased tear volume by 92-133%. Modulating the treatment with 50% duty cycle (3 seconds of stimulation repeated every 6 seconds) increased tear secretion an additional 23% above continuous stimulation. Tear secretion returned to baseline levels within several minutes of stimulation. Tear film osmolarity decreased by 7mOsmol/L, tear lipid increased by 24-36% and protein concentration increased by 48%. Relative abundance of most lacrimal gland proteins (i.e. lipophilin, lipocalin, prolactin-induced protein) remained the same, while several serum (i.e. serum albumin, IgG) and corneal (i.e. cytoplasmic-1 actin, transketolase, alpha-enolase and L-lactate dehydrogenase) proteins decreased with stimulation.
Conclusions: Electrical stimulation of the anterior ethmoid nerve increased aqueous tear volume, reduced tear osmolarity, added lipid and increased the concentration of normal tear proteins. Human studies with an intranasal stimulator should verify these results in patients with aqueous- and lipid-deficient forms of dry eye disease.
Commercial Relationships: Mark Brinton, None; **Andrea Kossler**, None; **Zara Patel**, None; **Jim Loudin**, 9440065 (P); **Manfred Franke**, 9440065 (P), Allergan (E); **Chris Ta**, None; **Daniel V. Palanker**, 9095723 (P)
Support: NIH Grant EY023259

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Presentation Time: 1:30 PM–3:15 PM
Tear film breakup time measurement based on corneal reflex interferences produced by dry spots
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Purpose: To test a new method for measuring the tear film breakup time based on interferences that appear on the corneal reflex. Dry spots in tear film cause abrupt height differences in the first eye surface, also the smoothness could be lost if corneal epithelium is exposed. Thus, the corneal reflex could present interferences caused by the phase differences, diffraction and speckle.
Methods: A setup for recording corneal reflex images was designed and built. In the illuminating path, a coherent lightsource (780nm laser diode) was projected onto the patient's cornea after reflection in a beamsplitter. The corneal reflection was recorded by means of a CCD camera after passing through the beamsplitter and an achromatic doublet lens (f'=50mm). This lens produced defocused (2D) images of the corneal reflex, making easier the interference detection. The breakup was determined detecting the appearance of small structures, produced by interferences due to the dry spots in the recorded corneal reflex images. The measurement consisted on positioning and centering the patient, then recording the corneal reflex in the time between two consecutive blinkings. The obtained images were compared to simulations, besides the breakup times

obtained with the proposed method and the conventional BUT method were contrasted.
Results: Ten subjects with mean (±SD) age of 26.7±3.4 years participated in the study. The corneal reflex images after the first blinking were uniform in all cases. With elapsing time, the image is degraded and interference patterns appeared as a consequence of dry spots. The appearance of the interference structures depended on the subject, being needed only a few seconds in some cases while in other cases it took up to 1 minute. These times were well correlated with the breakup time measured with the BUT method, although longer times were found in the proposed method. The experimental images were in good agreement with the simulations, in both cases similar interference structures were obtained.
Conclusions: A method for the break up measurement of the tear film, based on corneal reflex interferences caused by dry spots, has been presented. The method is noninvasive, objective, simple to use and low-cost. It is oriented to clinical practice and could be implemented in regular ophthalmic devices (double pass or Hartmann-Shack), as the configuration and optical elements of both setups are nearly the same.
Commercial Relationships: Mikel Aldaba, None; **Alejandro Mira Agudelo**, None; **Carlos E. Garcia-Guerra**, None; **John F. Barrera**, None; **Jaume Pujol**, None
Support: Spanish Ministry of Economy and Competitiveness DPI2014-56850-R; People Programme (Marie Curie Actions) of the Seventh Framework Programme of the European Union (FP7/2007-2013) under REA grant agreement no. 600388 (TECNIOspring programme), and the Agency for the Business Competitiveness of the Government of Catalonia, ACCIÓ.

Program Number: 472 **Poster Board Number:** A0397
Presentation Time: 1:30 PM–3:15 PM
Tear Film Break-Up: a molecular level view by employing in silico approach
Lukasz Cwiklik[1], Adéla Melcrová[1], Philippe Daull[2], Jean-Sebastien Garrigue[2]. [1]Institute of Physical Chemistry, Czech Academy of Sciences, Prague, Czech Republic; [2]Novagali Innovation Center, Santen SAS, Evry, France.
Purpose: Tear film breakup is assumed to be dependent on lipid composition of the tear film lipid layer (TFLL). However, both mechanism of the breakup and the role played by lipids are still not sufficiently known. This issue is particularly important for understanding tear film breakup in the case of dry eye where deficiencies of the TFLL often play a critical role. The macroscopically observed breakup is governed by the underlying molecular-level interactions between individual tear film components. We hypothesize that various lipid classes of TFLL play specific role during the onset of tear film breakup at the molecular level and hence have different role for maintaining tear film stability.
Methods: Molecular dynamics in silico simulations of the TFLL model in contact with aqueous subphase were performed. The model includes several types of polar and nonpolar lipids described employing the coarse grain MARTINI model. Microsecond-long simulations of laterally relaxed and compressed TFLL with varying polar lipids composition were performed.
Results: Deficiency of polar lipids in TFLL leads to poration of the polar monolayer and increased water-nonpolar lipids contacts. In contrast to the TFLL rich in polar lipids where cholesteryl esters partially incorporate in the polar monolayer and interact with water, in the deficient TFLL cholesteryl esters avoid interactions with the water subphase. Contacts between nonpolar triglycerides and the water phase are enhanced. In the case of laterally compressed and hence undulated TFLL, the diminished polar lipid content causes flattening of the water-lipid interface to minimize the interfacial tension. As observed in the laterally relaxed TFLL, cholesterol esters avoid contacts with water while triglyceride-water contact become more abundant.
Conclusions: The results obtained via in silico simulations support the hypothesis that involvement of various lipid classes differs during the breakup of the tear film deficient in polar lipids. Polar lipids form an incomplete monolayer with pores present. The pores are predominantly occupied by triglycerides involved in direct contacts with water while the presence of cholesteryl ester at the water-lipid boundary is diminished. Due to unfavorable water-triglyceride contacts, the system tends to reduce water-lipid interface which would lead to its breakdown at the macroscopic level.
Commercial Relationships: Lukasz Cwiklik, Santen SAS (R), Santen SAS (F); **Adéla Melcrová**, Santen SAS (F); **Philippe Daull**, Santen SAS (E); **Jean-Sebastien Garrigue**, Santen SAS (E)

Program Number: 473 **Poster Board Number:** A0398
Presentation Time: 1:30 PM–3:15 PM
Anti-inflammatory and Antioxidative Effects of *Camellia japonica* on Human Corneal Epithelial Cells and Experimental Dry Eye
Lian Cui[1, 2], Ying Li[1], Ji Suk Choi[1], Yung Hui Kim[1], Hyo Seok Lee[1], In Cheon You[3], Kyung Chul Yoon[1, 2]. [1]Department of Ophthalmology, Chonnam National University School and Hospital, Gwangju, Korea (the Republic of); [2]Department of Biomedical Sciences and Center for Creative Biomedical Scientists at Chonnam National University, Gwangju, Korea (the Republic of); [3]Department of Ophthalmology, Chonbuk National University Medical School and Hospital, Jeonju, Korea (the Republic of).
Purpose: To investigate the anti-inflammatory and anti-oxidative effects of *Camellia japonica* (CJ) on human corneal epithelial (HCE) cells and its therapeutic effects in a mouse model of experimental dry eye (EDE) after topical administration.
Methods: CJ extracts of varying concentrations (0.001%, 0.01%, and 0.1%) were used to treat HCE cells. Cell viability after treatment with CJ extracts was measured using the EZ-Cytox assay. The effects of CJ extracts on hydrogen peroxide (H_2O_2)-induced cytotoxicity in HCE cells were also measured. Dichloro-dihydro-fluorescein diacetate (DCF-DA) and dihydroethidium (DHE) assays were to analyze the anti-oxidative property of CJ extracts. Eye drops containing 0.001%, 0.01% or 0.1% CJ extracts, or a balanced salt solution (BSS), were applied to the EDE. Tear volume, tear film break-up time (TBUT), and corneal fluorescein staining scores were measured at 7 days after treatment. Levels of TNF-a, IL-1b, IL-6, IFN-r, CXCL-9, and CXCL-10 were measured with a multiplex immunobead assay. Production of reactive oxygen species (ROS) was also measured in the conjunctiva using the DCF-DA assay.
Results: The EZ-Cytox assay revealed that CJ extracts of varying concentrations were non-toxic to HCE cells. The viability of H_2O_2-treated HCE cells showed a significant improvement after pretreatment with 0.01% and 0.1% CJ extracts. In addition, treatment with 0.01% and 0.1% CJ extracts decreased ROS production in HCE cells. Mice treated with 0.1% CJ extracts showed significant improvements in tear volume, TBUT, and corneal fluorescein staining scores compared with the EDE and BSS groups. A significant decrease in the levels of inflammatory cytokines and chemokines was observed in the 0.01% and 0.1% CJ extracts groups. The levels of ROS significantly decreased in the 0.01% and 0.1% CJ extracts groups compared with the EDE and BSS groups.
Conclusions: CJ extracts improved the cellular viability and decreased ROS production in HCE cells. In addition, CJ extracts could improve clinical signs and reduce inflammatory and oxidative

stress markers in EDE, suggesting that topical CJ extracts could be used as a potential treatment agent for dry eye.
Commercial Relationships: Lian Cui, None; **Ying Li**, None; **Ji Suk Choi**, None; **Yung Hui Kim**, None; **Hyo Seok Lee**, None; **In Cheon You**, None; **Kyung Chul Yoon**, None

Program Number: 474 **Poster Board Number:** A0399
Presentation Time: 1:30 PM–3:15 PM
Therapeutic Efficacy of Topical Adiponectin-Derived Short Peptides and Globular Adiponectin for Experimental Dry Eye
Ying Li[1], Lian Cui[1], Hyo Seok Lee[1], Henry H. Hsu[4], Laszlo Otvos[2], Eva Surmacz[3], Kyung Chul Yoon[1]. [1]Department of ophthalmology, Chonnam National University Medical School & Hosp., Gwangju, Korea (the Republic of); [2]Olpe LLC, Audubon, PA; [3]Temple University, Philadelphia, PA; [4]Allysta Pharmaceuticals, Belmont, CA.
Purpose: To compare the therapeutic effect of topical adiponectin (ADP)-derived short peptides and globular adiponectin in a mouse model of experimental dry eye (EDE).
Methods: EDE was created by desiccating stress in 6- to 8-weeks old female C57BL/6 mice. Eye drops containing 0.01% globular ADP, 0.01% ADP peptide 399 or 355, or balanced salt solution (BSS) were applied. Tear volume, tear film break-up time (TBUT), and corneal staining scores were measured at 5 and 10 days after treatment. Levels of IL-1β, IL-6, IFN-γ, CXCL-9, and CXCL-10 were measured in the conjunctiva using a multiplex immunobead assay at 10 days. Flow cytometric analysis for CD4+CCR5+ T cells was also performed.
Results: The globular ADP and both ADP-derived short peptide groups showed a significant improvement in tear volume, TBUT, corneal staining scores, and CD4+CCR5+ T cell infiltration compared with the EDE control and BSS-treated groups. Significantly decreased levels of IL-1β, CXCL-9, and CXCL-10 were observed in the globular ADP group compared with the EDE control. The 0.01% ADP peptide 399 and 355 treatment groups showed significantly decreased levels of IFN-γ, IL-1β, IL-6, CXCL-9, and CXCL-10 compared with the EDE control group and IFN-γ, IL-6, and CXCL-9 compared with the BSS group. However, there were no significant differences in all clinical and experimental parameters among the globular ADP and both ADP-derived short peptide groups.
Conclusions: Topical application of ADP-derived peptides could improve clinical signs and decrease inflammation of the ocular surface of EDE, and the effects were similar to those of globular ADP.
Commercial Relationships: Ying Li, None; **Lian Cui**, None; **Hyo Seok Lee**, None; **Henry H. Hsu**; **Laszlo Otvos**, None; **Eva Surmacz**, None; **Kyung Chul Yoon**, None

Program Number: 475 **Poster Board Number:** A0400
Presentation Time: 1:30 PM–3:15 PM
Sleep Deprivation Compromises Lacrimal System and Homeostasis of the Ocular Surface
Wei Li[1], Sanming Li[1], Ke Ning[1], Jing Zhou[1], Yuli Guo[1], Houjian Zhang[1], Yu Zhu[1], Liying Zhang[1], Changkai Jia[1], Yongxiong Chen[1], Peter S. Reinach[2], ZUGUO LIU[1]. [1]Eye Inst & Xiamen Eye Ctr, Xiamen Univ Sch of Medicine, Xiamen, China; [2]Wenzhou Medical University, Wenzhou, China.
Purpose: Normal sleep plays a pivotal role in mental and physiological health of human being. Sleep deficiency and deprivation are associated with various systemic diseases such as obesity and cardiovascular disease. This study was conducted to investigate the effect of sleep deprivation on ocular surface tissues in a mouse model.
Methods:
Adult C57BL/6 mice were used to establish a sleep deprivation mouse model using modified "stick over water" method. Slit-lamp microscope observation, fluorescein test, and Schirmer's tear secretion test were performed to detect ocular surface and tear film change of the animals. Hematoxylin-Eosin, Oil Red O staining, cell apoptosis detection, immunofluorescence staining, Periodic Acid-Schiff (PAS) staining were performed on lacrimal gland and ocular surface tissues. LC/MS/MS analysis was performed to detect amino acids in lacrimal gland tissues. Quantitative real-time RT-PCR analysis was performed to detect gene expression in cornea and lacrimal gland.
Results: Mice exhibited aqueous tear secretion decrease, corneal epithelial defect and apoptosis, corneal squamous metaplasia, as well as hypertrophic changes of lacrimal gland acinar cells after sleep deprivation for different durations. Further investigation showed increased inflammatory cytokine expression, abnormal lipid metabolism and decreased protein synthesis of lacrimal gland after sleep deprivation.
Conclusions:
The pathological changes of mice after sleep deprivation mimic dry eye manifestations. These mice may be valuable model to study the pathophysiological process and mechanism of sleep disorder related ocular surface diseases.
Commercial Relationships: Wei Li, None; **Sanming Li**; **Ke Ning**, None; **Jing Zhou**, None; **Yuli Guo**, None; **Houjian Zhang**, None; **Yu Zhu**, None; **Liying Zhang**, None; **Changkai Jia**, None; **Yongxiong Chen**, None; **Peter S. Reinach**, None; **ZUGUO LIU**, None
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Program Number: 476 **Poster Board Number:** A0401
Presentation Time: 1:30 PM–3:15 PM
Stability study of SYL1001 eye drops (a siRNA compound) for Dry Eye Disease in different containers
Veronica Ruz, Yolanda Ruiz, Carlos Astrain, Carlos Segura, Noelia Miguel, Carlos millan, Victoria Gonzalez, Maria D. Company. Sylentis, Madrid, Spain.
Purpose: To study the stability of SYL1001 ophthalmic solution (a new chemical entity based on siRNA technology) as eye drops in different containers.
SYL1001 eye drops are a siRNA investigational new drug developed by Sylentis. SYL1001 has been developed to treat patients with Dry Eye Disease (DED). Currently the compound has completed Phase 2 of clinical development. The drug product has been formulated as an eye drops solution, in phosphate buffer saline, free of preservatives.
Methods: During the clinical trials corresponding to phases 1 and 2, and in order to select the best dose for the treatment of sign and symptoms of DED with SYL1001 eye drops, the solution was formulated in unit glass vials free of preservatives. Once the best dose was chosen, SYL1001 has been formulated in plastic ampoules in strips inside a foil to carry out phase 3 clinical trials and reach the market. Both types of primary pharmaceutical packaging have been compared in order to study the stability of the same formulation of SYL1001 drug product: unit-dose plastic containers (in strips inside a sachet) and in unit glass vials. These presentations have been tested under controlled stability conditions during 1 year with the aim to

assess the stability of the medication and comply with the regulatory requirements.
The samples were placed in qualified chambers protected from light at the following conditions: 5(±3) C, 25 (±2) C, and 40 C for plastic unit dose strips. Glass vials containers were placed at 5(±3) C condition. All samples were analyzed at different time-points. Physicochemical and microbiological tests were performed in order to assess that the product is within specifications.
Impurities of both investigational new products were also compared with the time zero analysis of the drug substance.
Results: All formulations showed good stability at the studied conditions during the time-points studied.
Conclusions: SYL1001 drug product shows good stability properties during the time of the study and is able to be packed in both type of containers and complies with the specifications that guarantee the integrity of the medication.
Commercial Relationships: Veronica Ruz, Sylentis (E); **Yolanda Ruiz**, Sylentis (E); **Carlos Astrain**, Sylentis (E); **Carlos Segura**, Sylentis (E); **Noelia Miguel**, Sylentis (E); **Carlos millan**, Sylentis (E); **Victoria Gonzalez**, Sylentis (E); **Maria D. Company**, Sylentis (E)

Program Number: 477 **Poster Board Number:** A0402
Presentation Time: 1:30 PM–3:15 PM
Treatment adherence and tear composition in diabetic and non-diabetic patients with dry eye syndrome
Rosa López-Pedrajas[1], Laura Armadans[1], Teresa Olivar[1], Jaime Beltrán[2], Fernando Llovet[2], María Miranda[1]. [1]Ciencias Biomédicas, Universidad CEU Cardenal Herrera, Valencia, Spain; [2]Clínica Baviera, Valencia, Spain.
Purpose: Dry eye syndrome (DES) is a multifactorial and complex disease with high prevalence among the population.
We have previously shown that protein concentration is decreased and malondialdehyde (MDA, a marker of lipid peroxidation) is increased in tears from elderly patients (Benlloch et al., 2013).
It is also known that, diabetic patients often display dry eye symptoms and decreased tear production, this fact could affect in some way, the disruption of visual function.
DES is usually treated with eye drops and treatment adherence is vital, because non-adherence could produce lesions and ulcers in the cornea, being these more difficult to treat.
The aim of this study was double: to evaluate the adherence to treatment in patients diagnosed with DES and to determine the differences in the composition of tears from non-diabetic and diabetic patients.
Methods: The study was adjusted to adhere to the requirements of Spanish law. A total of 115 subjects participated in this study. 83 patients answered a questionnaire about their use of DES medication. Tears from 16 non-diabetic (ND) and 16 diabetic (D) patients were collected with the help of a Schirmer strip. The total protein content of tears was measured by means of the Lowry method (Lowry et al., 1951) and MDA concentration was determined according to a modification of the method used by Richard et al. (1992).
Results: 74.70% of the patients that answered the questionnaire were women, and 63,86% of them were over 50 years of age. 22.89% of patients affirmed that they have decreased the use of the drugs for eye dryness in the last years because of the pricing of this product. Most patients did not know how or when to use this kind of products. There were no differences in the answers given by ND or D patients. Tears from D patients have a decreased total protein concentration, compared to tears obtained from ND patients. MDA concentration increased with age both in ND and D patients. ND women had higher MDA tear concentration that men, however, this difference was not observed in D patients.
Conclusions: Both ND and D patients showed a poor adherence to DES treatment. There are differences in the protein concentration in the tears from D patients when compared to ND patients. It is necessary to pay attention to the treatment adherence of this syndrome with special emphasis on D patients
Commercial Relationships: Rosa López-Pedrajas, None; **Laura Armadans**, None; **Teresa Olivar**, None; **Jaime Beltrán**, None; **Fernando Llovet**, None; **María Miranda**, None

Program Number: 478 **Poster Board Number:** A0403
Presentation Time: 1:30 PM–3:15 PM
Schirmer Strips Provide Reliable Tear-Production Rates
Clayton J. Radke[1, 3], Young Hyun Kim[2], Wing Li[2], Meng C. Lin[2, 3]. [1]Chemical Engineering, University of California, Berkeley, Berkeley, CA; [2]Clinical Research Center, School of Optometry, University of California, Berkeley, Berkeley, CA; [3]Vision Science Group, University of California, Berkeley, Berkeley, CA.
Purpose: Reliable measurement of tear production is critical to distinguish between aqueous deficient and evaporative dry eye. We show that Schirmer strip wetting lengths provide quantitative tear-production rates.
Methods: In a standard Schirmer tear test (STT), tear production after 5 min of eye closure is classified as "normal" when the strip wetted length is more than 10 mm, "deficient" or "dry eye" if less than 5 mm, and "equivocal" when lying between (Cassen et al., 1997). With this procedure, it is not possible to predict quantitative tear production. For quantitative analysis, measurement of wetting length is required at several time points.
When SST wetted length is measured as a function of time, a linear increase occurs after an initial nonlinear period (Holly et al., 1997) (see Figure 1 from Clinch et al, 1983). Upon careful analysis of STT wetting kinetics, we identify the initial nonlinear wetting period as due to local depletion of tear in the lid margin followed by a slower linear time regime where exhaustion of the lid-margin tear slows tear supply. Our analysis yields the simple result that volumetric tear-production rate (in μL/min) is calculated from the slope of linear portion of wetting kinetics multiplied by the cross-sectional area of the Schirmer strip (strip width times thickness). By measuring the time course of the wetting front, quantitative tear-production rate is assessed.
Results: From the linear slope in Figure 1 and the described calculation, we obtain an average tear-production rate of 1.7 μL/min for 50 subjects with no apparent ocular surface disease, a realistic value. For best clinical results, we suggest that subject eyes be anesthetized and the Schirmer strip be sheathed to minimize evaporation. We also recommend utilizing dyed Schirmer strips with mm markings and recording at least three wetting lengths at, for example, 3, 4, and 5 min.
Conclusions: We rigorously establish tear-wetting kinetics in a Schirmer strip and present a simple methodology to obtain quantitative tear-production rate.

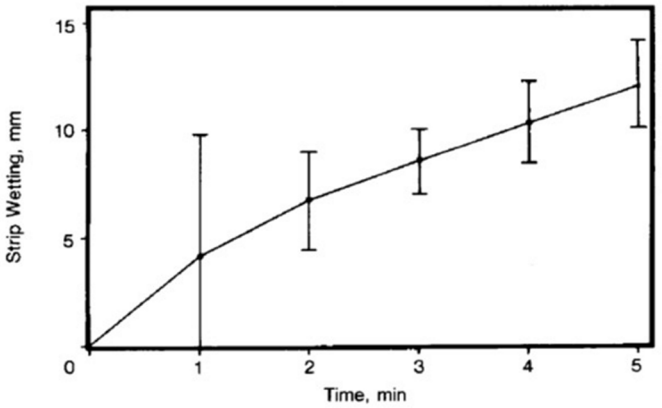


Figure 1. STT average wetted length for 50 human subjects under local anesthetic as a function of time (Clinch et al., 1983). Solid line connects datum points. Standard deviation error bars are shown.
Commercial Relationships: Clayton J. Radke, None; **Young Hyun Kim**, None; **Wing Li**, None; **Meng C. Lin**, None

Program Number: 479 **Poster Board Number:** A0404
Presentation Time: 1:30 PM–3:15 PM
Acute corneal epithelial debridement in rats unmasks the corneal stromal nerve responses to ocular stimulation: Implications for abnormal sensations in recurrent corneal erosion and dry eye disease
Harumitsu Hirata[1], Kamila Mizerska[1], Valentina Dallacasagrande[1], Victor H. Guaiquil[2], Mark Rosenblatt[2]. [1]Ophthalmology, Weill Cornell Medical College, New York, NY; [2]Department of Ophthalmology and Visual Sciences, University of Illinois-Chicag, Chicage, IL.
Purpose: It is widely accepted that the mechanisms for transducing sensory information reside in the nerve terminals (NTs). Occasionally, however, studies have appeared demonstrating that similar mechanisms may exist in the axon to which these NTs are connected. We examined this issue using the cornea where NTs in the epithelial cell layers are easily accessible for debridement, leaving the underlying stromal (axonal) nerves (SNs) undisturbed.
Methods: In isoflurane-anesthetized rats, we recorded extracellularly from single trigeminal ganglion neurons innervating the cornea. Two types of corneal neurons, low threshold cold-sensitive plus dry sensitive (LT-CS+DS) and high threshold cold-sensitive plus dry sensitive (HT-CS+DS) neurons, were studied in response to a variety of ocular stimuli before and after the epithelial debridement. We previously hypothesized that these neurons play a critical role in tearing and ocular pain. Immunohistochemical techniques were also used to confirm the extent and the consistency of the debridement lesions.
Results: We found that the responses in both types of neurons to dryness, wetness, and menthol stimuli applied to the ocular surface were completely abolished by epithelial debridement, while a significant amount of the responses to the cold, heat and hyperosmolar stimuli (HOS) in LT-CS+DS neurons (but not HT-CS+DS neurons) still remained even after debriding the areas of the cornea that far exceeded the neurons' receptive fields. Surprisingly, the responses to heat in ~ half of the neurons tested were augmented after the debridement. We were also able to evoke these residual responses and follow the trajectory of the SNs, which we subsequently confirmed histologically. The residual responses always disappeared when the the limbus, where the SNs originate, was cut.
Conclusions: In addition to the transduction mechanisms in the NTs giving rise to the responses to ocular dryness and menthol, this study provides strong evidence that the additional transduction mechanisms for the sensory modalities, such as cold, heat and HOS responses, originate in SNs. The functional significance of these residual and enhanced responses from SNs may be related to the abnormal sensations observed in a variety of ocular disease and symptoms.
Commercial Relationships: Harumitsu Hirata; **Kamila Mizerska**, None; **Valentina Dallacasagrande**, None; **Victor H. Guaiquil**, None; **Mark Rosenblatt**, None
Support: NIH Grants EY023555, EY018594, and the Research to Prevent Blindness Grants to Department of Ophthalmology, Weill Cornell Medical College.

Program Number: 480 **Poster Board Number:** A0405
Presentation Time: 1:30 PM–3:15 PM
Correlations of mRNA expression profiles with clinical symptoms and signs in conjunctival imprints from Sjögren's syndrome patients
Hong Liang[2, 1], Karima Kessal[2, 1], Ghislaine Rabut[1], Philippe Daull[3], Jean-Sebastien Garrigue[3], Mylène DOCQUIER[4], Stéphane MELIK PARSADANIANTZ[2, 5], Françoise Brignole-Baudouin[2, 6], Christophe Baudouin[2]. [1]Centre Hospitalier National d'Ophtalmologie des Quinze-Vingts, Paris, France; [2]Institut de la vision, UPMC Univ Paris 06, Paris, France; [3]R&D, SANTEN SAS, Evry, France; [4]iGE3 Genomics Platform, University of Geneva, Geneva, Switzerland; [5]CNRS, UMR_7210, Paris, France; [6]Faculté de Pharmacie, Université Paris Descartes, Paris, France.
Purpose: In dry eye disease, symptoms and signs are not always correlated. The aim of this study was to characterize the expressions of inflammation-related genes on the ocular surface of Sjögren's syndrome (SS) patients and to evaluate which of them could be correlated with clinical symptoms and signs.
Methods: The study was conducted on 30 patients with SS dry eye patients and 15 healthy controls. A set of clinical signs and symptoms were evaluated using the ocular surface disease index (OSDI) questionnaire, corneal fluorescein staining (CFS), tear breakup time (TBUT), Schirmer's test and tear osmolarity. Conjunctival superficial cells were collected by impression cytology and total RNAs were extracted and the transcripts were analyzed using nCounter® human inflammation code set of 249 genes (NanoString technologies). The fold change of mean with a cut off ≥ 1.5 and <0.8 was chosen to select the differentially expressed genes in the two groups. Mann-Whitney non-parametric statistical test was used, and $p<0.05$ was considered as statistically significant. A Spearman correlation was performed to correlate the up and down regulated genes with the clinical exams.
Results: Twenty-seven genes were up-regulated and 13 were down-regulated with significant fold changes ranging from 16.7 to 1.5 and 0.8 to 0.3 respectively. OSDI and CFS were the most significantly correlated with 21 and 19 inflammatory genes, respectively. More interestingly, among all the up-regulated genes, 12 genes including IL-6, CCL24, CCL22, CCL5, CCR1, C2, C1QB, CFB, STAT1, CXCR4, LY96, and HLA-DRA were positively correlated with both OSDI and CFS. Two down-regulated genes (GNGT1, HSPB2) were negatively correlated with OSDI and CFS. CXCL2, CXCL9, CXCL10, NOS2, NOX1, MAFF and NOD2 were correlated exclusively with OSDI, and CCL4, TNF, IL-15, ITGB2 and HLA-DRB1, only with CFS. IL1RN was the only gene positively correlated with the Schirmer test. Furthermore, no gene was found correlated with T-BUT or tears osmolarity.
Conclusions: These results highlight the relationship between the expressed inflammatory genes and the patient's symptoms and signs, assessed with OSDI and CFS respectively. The analysis of

inflammatory genes implicated in SS-associated dry eye could be an important tool to determine a pathophysiological profile potentially usable as a biomarker of the disease.
Commercial Relationships: Hong Liang, SANTEN SAS (P); **Karima Kessal**, SANTEN SAS (P); **Ghislaine Rabut**, None; **Philippe Daull**, SANTEN SAS (E); **Jean-Sebastien Garrigue**, SANTEN SAS (E); **Mylène DOCQUIER**, None; **Stéphane MELIK PARSADANIANTZ**, None; **Françoise Brignole-Baudouin**, None; **Christophe Baudouin**, SANTEN SAS (C), Allergan (C), Alcon (C), Théa (C)

Program Number: 481 **Poster Board Number:** A0406
Presentation Time: 1:30 PM–3:15 PM
In Vitro and In Vivo correlation between diadenosine tetraphosphate and osmolarity in Sjögren Syndrome patients
Basilio Colligris[1, 2], Gonzalo Carracedo[1], Rodriguez Candela[1], Maria J. Perez de Lara[2], Ana Guzman-Aranguez[2], Jesus J. Pintor[2]. [1]University Complutense of Madrid, Madrid, Spain; [2]Biochemistry and Molecular Biology IV, University Complutense of Madrid, Madrid, Spain.
Purpose: To evaluate the correlation between osmolarity, concentrations of diadenosine tetraphosphate (Ap_4A) and matrix metalloproteinase 9 (MMP-9) in Sjögren Syndrome patients compared with healthy subjects.
Methods: Twelve patients of primary Sjögren Syndrome (46.64 ± 13.34 years) and twenty healthy volunteers (41.38 ± 9.67 years) participated in the study. All participants in the study were women. Osmolarity, Ap_4A and MMP-9 concentration, Schirmer test, Tear film break up time (TFBUT) and OSDI questionnaire were evaluated. To perform *In Vitro* assays, human conjunctival cells were incubated overnight in two different osmotic solutions (290 mOsm/L and 320 mOS/L) and supernatants were collected. Diadenosine tetraphosphate and MMP-9 concentration were evaluated for each solution.
Results: Dry eye symptomatology, osmolarity and Ap_4A were higher in Sjögren Syndrome group than in control group ($p<0.05$). In contrary, MMP-9 showed a non-significant difference between both groups ($p=0.244$). A positive correlation between osmolarity and Ap_4A was found ($R= 0.451$; $p=0.016$). Tear volume and TFBUT were statistically lower in Sjögren Patients than healthy subjects ($p<0.05$). In Vitro assays showed that Ap_4A was statistically higher (two-folds) in the hyperosmotic solution, presenting a strong correlation between Ap_4A and osmolarity ($R=0.984$; $p < 0.001$)
Conclusions: Osmolarity and diadenosine tetraphosphate have a positive correlation in both studies performed, *In Vivo* and *In Vitro*. The relationship between these objective markers of dry eye seems to corroborate the diagnostic role of Ap_4A in the Sjögren Syndrome.
Commercial Relationships: Basilio Colligris, None; **Gonzalo Carracedo**, None; **Rodriguez Candela**, None; **Maria J. Perez de Lara**, None; **Ana Guzman-Aranguez**, None; **Jesus J. Pintor**, None
Support: SAF2013-44416-R, RETICS RD12/0034/0003

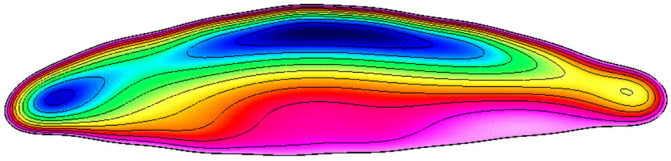
Program Number: 482 **Poster Board Number:** A0407
Presentation Time: 1:30 PM–3:15 PM
INTERDISCIPLINARY EVALUATION OF PATIENTS WITH PRIMARY SJÖGREN'S SYNDROME
Behzod Tashbayev[1], Shermin Rusthen[2], Xianjun Chen[1], Ø Utheim[1], Alix Young[2], Bente Herlofson[2], Tor Paaske Utheim[3, 1], Janicke Cecilie Liaaen Jensen[2]. [1]The Norwegian Dry Eye Clinic, Oslo, Norway; [2]Institute of Clinical Dentistry, Faculty of Dentistry, University of Oslo, Oslo, Norway; [3]Institute of Oral Biology, Faculty of Dentistry, University of Oslo, Oslo, Norway.
Purpose: *To carry out comprehensive comparative examination of a Norwegian cohort of patients with primary Sjögren's syndrome (pSS) and age- and gender-matched controls.*
Methods: Thirty-four female patients with pSS (age 53±12) and 32 controls (age 49±11.5) were recruited to the study. All participants underwent comprehensive dry eye and dry mouth examinations. Ophthalmological examinations included McMonnies and Ocular Surface Disease Index (OSDI) questionnaires, and clinical dry eye tests – tear osmolarity, Schirmer I test, tear film break-up time (TFBUT), ocular surface staining (OSS) and meibomian glands drop-out assessed with meibography. Dry mouth examinations included the summated xerostomia inventory (SXI) and clinical oral dryness score (CODS). Secretion rate of unstimulated (UWS) and stimulated whole saliva (UWS) was recorded. Mann-Whitney U test was used for inter-group comparisons, while Spearmans rank correlation was applied to search for possible correlations.
Results: Compared to the control group, pSS patients had higher McMonnies (17.6±3.8 vs 4.1±2.0, $p<0.01$) and OSDI scores (34.8±19.2 vs 4.8±7.5, $p<0.01$), higher tear osmolarity level (334.8±21.6 vs 319.7±15.8, $p<0,01$) lower Schirmer I value (4.8±4.0 vs 16.2±11.6, $p<0.01$), shorter TFBUT (2.4±2.6 vs 5.4±3.3, $p<0,01$) and higher OSS (3.9±2.3 vs 0.8±1.2, $p<0.01$). Additionally, the pSS group represented with higher level of meibomian gland drop out score in the upper eyelids (1.8±0.9 vs 1.4±0.6, $p=0.043$) and the lower eyelids (1.4±0.7 vs 1.1±0.3, $p=0.006$). Patients with pSS also demonstrated more severe oral dryness as shown by higher mean SXI score (12.1±2.5 vs 5.9±1.0, $p<0.01$), CODS (4.9±2.0 vs 0.6±0.9, $p<0.01$), decreased levels of UWS (0.07±0.07 vs 0.29±0.17 ml/min, $p<0.01$) and SWS (0.58±0.40 vs 1.49±0.67 ml/min, $p<0.01$). Moderate correlations between SWS and Schirmer I test ($r=0.56$, $p=0.03$), SXI and McMonnies questionnaires ($r=0.456$, $p=0.04$) were observed.
Conclusions: The findings of the current study revealed positive correlations between dry eye and dry mouth symptoms as well as lacrimal and salivary secretion underlining that interdisciplinary evaluation of pSS patients provides better understanding of the disease.
Commercial Relationships: Behzod Tashbayev, None; **Shermin Rusthen**, None; **Xianjun Chen**, None; **Øygunn Utheim**, None; **Alix Young**, None; **Bente Herlofson**, None; **Tor Paaske Utheim**, None; **Janicke Cecilie Liaaen Jensen**, None

Program Number: 483 **Poster Board Number:** A0408
Presentation Time: 1:30 PM–3:15 PM
SNP Variation in IL10, TNF-α and TNFAIP3 Genes in Patients with Dry Eye Syndrome and Sjogren's Syndrome
Abraham Solomon, Hadas Ben Eli, Nir Gomel, Rania Abu Seir, Riki Perlman, Eldad Ben Chetrit, Dror Mevorach, Geffen Kleinstern, Doron Aframian, Ora Paltiel. Hadassah-Hebrew University, Jerusalem, Israel.
Purpose: Cytokine-related genes are assumed to be key players in dry eye syndrome (DES) and Sjogren's syndrome (SS) pathogenesis. However the association between specific genes variants and both DES and SS are unclear, and comparisons between these two diseases has not yet been performed. In this study we compared single nucleotide polymorphism (SNP) variation in genes encoding cytokine levels among SS and DES patients in Israel.
Methods: A total of 180 subjects were recruited, 82 with SS and 98 with DES. Using a candidate gene approach and allele-specific PCR technique for genotyping, the proportions of risk alleles in TNFα (rs1800629), IL10 (rs1800896) and TNFAIP3 (rs2230926) SNPs were compared between study groups.

Results: The allelic distribution of the study groups was found to be very similar and match to Caucasians (CEU – Northern Europeans from Utah) population distributions in these SNPs. While none of the SNPs variants were found to be statistically significant associated to SS or DES in a recessive model, in an additive model the TNFα (rs1800629)-G risk allele was found among a higher proportion of SS patients compared to DES (Homozygote-G: 70.8% vs. 64.7%; Heterozygote: 26.9% vs. 11.2%, respectively, p=0.02). After adjusting for possible confounders, none of the tested SNPs were associated with SS compared to DES.
Conclusions: The frequency of IL10 (rs1800896-A) and TNFAIP3 (rs2230926-G) alleles was not found differ significantly between SS and DES patients. These findings may be due to limited power of the sample size of 180 participants. The TNFa (rs1800629-G) SNP seems to be associated with SS in an additive model. TNFa protein levels are known to be associated with inflammation, outcome of infection, and susceptibility to autoimmune diseases such as SS. The gene has also been associated with non-Hodgkin lymphoma, a serious complication of SS. Further comparison to healthy controls is required, as well as exploring other SNPs variants relating to the immune pathway in order to understand the genetic basis of DES and SS etiology.
Commercial Relationships: Abraham Solomon, None; **Hadas Ben Eli**, None; **Nir Gomel**, None; **Rania Abu Seir**, None; **Riki Perlman**, None; **Eldad Ben Chetrit**, None; **Dror Mevorach**, None; **Geffen Kleinstern**, None; **Doron Aframian**, None; **Ora Paltiel**, None
Support: "Israeli Cancer Association" (grant number: 8037815) and the MERC-USAID (grant number: 8037812)

Program Number: 484 **Poster Board Number:** A0409
Presentation Time: 1:30 PM–3:15 PM
Ocular tolerability of a ciclosporin eye drop based on PAD™ technology
Frederic Gomez, Morten Praestegaard, Fraser Steele. MC2 Therapeutics, Hoersholm, Denmark.
Purpose: MC2 Therapeutics is developing PADciclo™, a new and optimized formulation of ciclosporin (CsA) based on PAD™ technology currently in clinical development for the treatment of moderate to severe dry eye. This new formulation contains less surfactants and is aimed to improve tolerability of CsA in dry eye. In these studies, ocular tolerance of PADciclo™ was determined up to 6 months in New Zealand White albinos rabbits.
Methods: The PADciclo™ eye drop targets ocular inflammation which is well-known as one of the underlying causes of dry eye. Two studies were conducted. In a 28-day ocular tolerance study, animals received four ocular administrations per day of PADciclo™ vehicle or PADciclo™ 0.1% CsA, separated by intervals of approximately three hours. In a 6-month ocular tolerance study, animals received two ocular administrations per day of PADciclo™ vehicle or PADciclo™ 0.1% CsA. For both studies, clinical signs, corneal sensitivity, body weights and food and water consumption were monitored.
Results: This is the first time that PADciclo™ tolerance data in animals are reported. PADciclo™ 0.1% CsA and PADciclo™ vehicle administered 4 times daily for 28 days or 2 times daily for 26 weeks in albino rabbits were macroscopically and microscopically very well tolerated. There were no treatment- or administration-related effects on body weight, clinical observations, food consumption, ophthalmic examinations, macroscopic observations at necropsy, or histopathology.
Conclusions: The presented studies confirm that the PADciclo™ vehicle and PADciclo™ 0.1% CsA administered 4 times daily for 28 days or 2 times daily for 26 weeks is safe and very well tolerated by the rabbit eye.
Commercial Relationships: Frederic Gomez, MC2 Therapeutics (C); **Morten Praestegaard**, MC2 Therapeutics (E); **Fraser Steele**, MC2 Therapeutics (E)

Program Number: 485 **Poster Board Number:** A0410
Presentation Time: 1:30 PM–3:15 PM
THE INFLUENCE OF TEAR SUPPLY ON TEAR FILM FORMATION DURING THE UPSTROKE
Kara Maki[1], William Henshaw[2], Alex McManus[1], Richard J. Braun[3], Tobin Driscoll[3]. [1]School of Mathematical Sciences, Rochester Institute of Technology, Rochester, NY; [2]Department of Mathematical Sciences, Rensselaer Polytechnic Institute, Troy, NY; [3]Department of Mathematical Sciences, University of Delaware, Newark, DE.
Purpose: The purpose of this study is to examine the influence of the tear supply on the formation of the tear film. During the upstroke, tear fluid from the upper meniscus and from under the upper lid is distributed onto the ocular surface to form a stable tear film. Because the current state-of-the-art instrumentation does not yet have the capability to estimate the tear film thickness over the entire front of the eye during the upstroke, especially near the upper lid, it is not fully understood how the tear supply impacts the tear film formation, and thus subsequent tear film thinning during the interblink.
Methods: In this study, we explore a mathematical model to simulate the tear film thickness on a realistic moving eye-shaped domain. The domain is described by curves fit to lid margins in a video of a blinking eye. The motion of the tear film is influenced by viscosity and surface tension. We examine how different models of tear supply, linked to lid motion and lid speed, affect the tear formulation and subsequent tear film breakup times.
Results: We find the formation of the tear film during the upstroke is sensitive to the lid motion, lid shape, and tear supply from under lids. Our results will be compared and contrasted with prior one-dimensional modeling efforts as well as experimental observations.
Conclusions: A simulation of the tear film dynamics on an eye-shaped domain was created to study the influence of the tear supply on tear film formation during a blink was studied. Our model provides insight into tear film dynamics near the upper lid, and over the exposed ocular surface, during a blink.



A snapshot of the tear film thickness profile during the upstroke where blue indicates thin regions and pink thick regions.
Commercial Relationships: Kara Maki, None; **William Henshaw**, None; **Alex McManus**, None; **Richard J. Braun**, None; **Tobin Driscoll**, None
Support: NSF 1412141

Program Number: 486 **Poster Board Number:** A0411
Presentation Time: 1:30 PM–3:15 PM
Detection of sex steroids and their metabolites in human tears using LC-MS/MS
Emma Gibson[1], Martin Bucknall[1, 3], Blanka Golebiowski[1], James Wolffsohn[2], Fiona Stapleton[1]. [1]School of Optometry and Vision Science, UNSW, Sydney, NSW, Australia; [2]School of Life and Health Sciences, Aston University, Birmingham, United Kingdom; [3]Bioanalytical Mass Spectrometry Facility, UNSW, Sydney, NSW, Australia.
Purpose: Detection of sex steroids in tears is problematic due to low available volumes of tears and trace level analyte concentrations. This study aimed to develop a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to detect sex steroids in human tears.
Methods: Limits of detection were determined for a series of sex steroids and metabolites in a mixture of standards and this technique was subsequently applied to human tear extracts. Fifteen analytes were analysed in mixtures at concentrations of 0.1, 0.3, 1, 3, 10, 30, 100 and 1000 ng/mL per component. Chromatographic separation was achieved on a Waters Acuity BEH UPLC C18 column (1.7µm, 1mmx150mm) using an acetonitrile-water gradient. Both water and acetonitrile mobile phases contained 10mM ammonium formate. Analysis was performed using alternating positive and negative atmospheric-pressure chemical ionisation (APCI) with selected-ion monitoring (SIM).
Basal tears were collected and pooled from 4 healthy pre-menopausal females (total 100µl). To precipitate protein, cold ethanol was added to tears at a ratio of 6:1 and centrifuged at -2°C for 25 minutes at 18,000g. 100µl of supernatant was removed for analysis (diluted tears). A further 400µl of supernatant was reduced in a vacuum concentrator to 80µl (concentrated tears). Both tear extracts were then analysed as described above.
Results: Both positive and negative ionisation modes were used because single ion mode analysis was not optimal for all analytes. Limits of detection (Signal/Noise > 3:1) for standards on column (10 µl injected) for negative ionisation were: 3α-Diol-G 30pg, ADT-G 30pg, DHEA-S 50pg, 2-hydroxyestradiol 80pg, 2-hydroxyestrone 80pg. For positive ionisation: estriol 30pg, 16-hydroxyestrone 8pg, estradiol 800pg, testosterone & DHEA 100pg total, estrone 15pg, androstenedione 2pg, androsterone 70pg, DHT 8pg, progesterone 0.8pg. DHEA and testosterone are chemically similar and could not be separated. Progesterone, ADT-G and 3α-Diol-G were successfully detected in concentrated tear extract.
Conclusions: A partly successful LC-MS/MS method to measure sex steroids, metabolites and precursors was developed. One sex steroid and two metabolites were detected in concentrated human tears. Further elaboration of these techniques will enable detection of additional analytes in tears. This data shows that LC-MS/MS can detect certain sex steroids and metabolites in concentrated human tear extract.
Commercial Relationships: Emma Gibson, None; **Martin Bucknall**, None; **Blanka Golebiowski**, None; **James Wolffsohn**, None; **Fiona Stapleton**, None

Program Number: 487 **Poster Board Number:** A0412
Presentation Time: 1:30 PM–3:15 PM
Hyaluronic Acid Preparation with Improved Corneal Wettability Property
Danilo Aleo[1], Barbara Melilli[1], Maria G. Saita[1], Assunta Borzacchiello[2], Sergio Mangiafico[1], Melina Cro[1], Sebastiano Mangiafico[1]. [1]R&D, Medivis, Catania, Italy; [2]IPCB, National Research Council, Napoli, Italy.
Purpose: The wettablility of corneal surface is a very important characteristic of tear substitutes. It is usually obtained with the use of surfactants, substances with very poor ocular tolerability. The aim of our study was to develop a new association between hyaluronic acid (HA) and polivinylalcohol (PVA), polymer able to increase the wettability property of the HA formulation without modifying the rheological characteristics of HA.
Methods: Ophthalmic hypotonic (150 mOsm/l) preparation of HA 0.20% (SVS201) was added with 0.25% of PVA (MDV200). Corneal wettability property of the two formulations, MDV200 and SVS201, was evaluated by the measurement of contact angle conducted 5 times for each formulation as function of time by OCAE15 System. Comparison between MDV200 and SVS201 was done by evaluating the contact angle measured after 50 sec. Plexiglass and polyamide having a surface tension of 38-40mN/m were used as models of the corneal surface (40mN/m).
Rheological characteristics were measured with Bohlin CVO Rehometer, using a cone-and-plate geometry (CP60/2); elastic modules (G') and viscous modules (G'') were evaluated as function of frequency, between 0.1-10.0Hz.
Results: MDV200 and SVS201 maintained the same viscosity-shear rate dependence as well as the elastic (G') and the viscous (G'') modules, between 0.1-10.0Hz.
MDV200 showed a very low contact angle (57°) when compared with the formulation without PVA (SVS201, 81°) when polyamide surface was used. When plexiglass was used, values were: MDV200 (64°) and SVS 201 (69°).
Conclusions: PVA was able to decrease the contact angle in the plexiglass surface as well as in polyamide. The effect was higher in polyamide than in plexiglass, they act like the cornea: are hydrophobic and have very similar surface tension. However, due to the presence of nitrogen and oxygen atoms in its molecular structure, polyamide acts more like the cornea and differently to the plexiglass. In other words polyamide is able, just like the corneal tissue, to interact with MDV200 through the formation of hydrogen bonds. We may then conclude that polyamide is better experimental model than plexiglass, and that MDV200, due to the presence of PVA, has a potential much higher corneal wettability (lower contact angle) than pure HA formulation (SVS201).
Commercial Relationships: Danilo Aleo, Medivis (E); **Barbara Melilli**, Medivis (E); **Maria G. Saita**, Medivis (E); **Assunta Borzacchiello**, CNR (E); **Sergio Mangiafico**, Medivis (E); **Melina Cro**, Medivis (E); **Sebastiano Mangiafico**, Medivis (E)

Program Number: 488 **Poster Board Number:** A0413
Presentation Time: 1:30 PM–3:15 PM
Secretory phospholipase A2-IIA (sPLA2-IIA) activity and concentration in human tears and correlation with the ocular surface disease index (OSDI) scores
Shyam Panthi, Jason J. Nichols. Vision Science, University of Alabama at Birmingham, Birmingham, AL.
Purpose: Secretory phospholipase A2 (sPLA2) is a potent antimicrobial enzyme found in the tear film that protects the ocular surface from microbial pathogens. The enzyme may also play a role in the degradation of tear phospholipids, which might cause lipid layer disruption and tear instability. Increased sPLA2 activity may also lead to increased formation of pro-inflammatory lipid mediators responsible for ocular surface inflammation. To our knowledge, the enzyme's activity and concentration have not been correlated with patient-reported outcomes. Our purpose was to evaluate the potential relation between the activity and concentration of sPLA2-IIA and subjective outcomes.

Methods: Ten µL of tears were collected from each eye of 20 normal subjects using glass microcapillaries, pooled, and stored at -80 °C until analysis. sPLA2-IIA activity was calculated using 1,2-dithio analog of diheptanoyl phosphatidylcholine as substrate. A double-antibody sandwich ELISA was used to determine the sPLA2-IIA concentrations. Subjects also completed the Ocular Surface Disease Index (OSDI) which was scored per recommended guidelines. The activity and concentration were then correlated with the OSDI using Spearman's rank correlation coefficient. A p-value of less than 0.05 was considered significant.
Results: The mean sPLA2-IIA activity in normal human tears was found to be 0.014 ± 0.028 µmol/min/ml while the mean sPLA2-IIA concentration was found to be 8111.098 ± 7.662 pg/ml. A moderate but non-significant negative correlation was found between the sPLA2-IIA activity and OSDI scores (ρ= - 0.42, p=0.06). sPLA2-IIA concentration had a strong negative correlation with the OSDI scores (ρ=-0.67, p=0.001).
Conclusions: The results indicate that tears contain low activity and concentration of sPLA2-IIA which may be due to the fact that all the tear samples came from normal individuals. The significant negative correlation between OSDI scores and the sPLA2-IIA concentration suggests increased activity in the tears may protect the ocular surface, thereby leading to lower subjective scores.
Commercial Relationships: Shyam Panthi, None; **Jason J. Nichols**, None

Program Number: 489 **Poster Board Number:** A0414
Presentation Time: 1:30 PM–3:15 PM
Nitration reduces lactoferrin antibacterial activity
Amani Alhalwani[1, 2], John E. Repine[2], J. A. Huffman[1]. [1]Chemistry and biochemistry, University of Denver, Englewood, CO; [2]Webb-Waring Center, University of Colorado Denver-School of Medicine, Aurora, CO.
Purpose: Lactoferrin (LF), a prominent component of tears and eye tissue, is a multifunctional protein that is a key contributor to ocular health and disease. Perturbations of LF develop in a variety of eye conditions such as infection, dry eye syndrome, and Sjögren's Syndrome and, accordingly, may impact ocular function in many ways. LF perturbations undoubtedly occur as a consequence of ocular inflammation and exposure to environmental urban air pollutants, most notably, nitrating molecules, such as nitrogen oxide, that can nitrate tyrosine in proteins, like LF, on the surface of the eye. Because one important function of LF is antibacterial activity, we hypothesized that inflammation and environmental pollution inducing nitrating reactions could decrease the antibacterial activity of LF and contribute to ocular infection, dry eye syndrome, Sjorgren's syndrome, and other ocular disorders. The specific aim of our study was to investigate the effect of nitration on the chemical structure and antibacterial function of lactoferrin.
Methods: Human lactoferrin (LF) was nitrated using two nitrating agents: tetranitromethane (TNM) mixed with methanol in a Tris-HCl buffer was used as a surrogate exogenous nitrating agent; peroxynitrite ($ONOO^-$) in PBS buffer was used as a surrogate endogenous nitrating agent. LF chemical properties were then determined using absorbance spectroscopy, fluorescence, and SDS-PAGE. Subsequently, NLF concentrations were measured using a sandwich ELISA assay recently developed in our laboratory. Antimicrobial activity against *Escherichia coli* and *Entercococcus faecalis* of NLF was evaluated using the standard agar plate inhibition test.
Results: We found that reaction with TNM and $ONOO^-$ changed the fundamental chemical properties of LF. Absorbance spectroscopy of LF revealed a peak at 280 nm, while, in contrast, following nitration, the absorbance peak of NLF increases to 350 nm. NLF also had a reduced fluorescence intensity compared to LF. Finally, NLF had reduced antibacterial activity compared to LF.
Conclusions: Nitration of lactoferrin produced in aqueous solution using TNM and $ONOO^-$ changed the chemical structure and reduced the antimicrobial activity of LF. Our results suggest that nitration of LF by exposure to inflammation and environmental nitrosative oxidants may contribute to ocular disorders such as infection, dry eye syndrome, and Sjögren's Syndrome by reducing LF antibacterial and other protective activities.
Commercial Relationships: Amani Alhalwani; **John E. Repine**, None; **J. A. Huffman**, None
Support: Knoebel Center for the Study of Aging

Program Number: 490 **Poster Board Number:** A0415
Presentation Time: 1:30 PM–3:15 PM
Recovery of the inflammatory response and ocular surface change after termination of short-term exposure keratopathy: A rabbit eye model
Wei-Li Chen, Lily Chen, Wen-Hui Tu, Fung-Rong Hu. Ophthalmology, National Taiwan University Hospital, Taipei, Taiwan.
Purpose: Despite the clinical importance of evaporative dry eye, the inflammatory response and structural change that occur on the ocular surface in exposure keratopathy are not well understood. We hypothesized that the involvement of inflammatory cells, cytokines, and ocular epithelial pathologies in short term exposure keratopathy would result in a long recovery period post exposure termination.
Methods: A short term exposure keratopathy model in New Zealand white rabbits was made by opening the right eyelids for 4 hours(h), then discontinuing the exposure for 0h, 4h, 8h and 24h (N=3x 4 groups=12). Ultrasound pachymetry and HRT in vivo confocal microscopy were used to detect central total corneal thickness, while in vivo confocal microscopy and impression cytology were used to evaluate the morphology of ocular surface epithelium and the infiltration of inflammatory cells. At 0h, 4h, 8h and 24h after discontinuation of exposure, immunohistochemistry was performed to stain for macrophages, neutrophils, CD4(+)T cells and CD8(+) T cells. Ocular surface change was detected via scanning electron microscopy (SEM). Tear film concentrations of IL-1, IL-2, IL-8, IL-17 and TNF-α were analyzed by multiplex immunobead assay.
Results: After discontinuation of eye exposure, recovery of corneal thickness was found within 4h. In vivo confocal microscopy showed no morphological change in the peripheral corneal, limbal and perilimbal conjunctival epithelia at all examined time points. Time-dependent decrease in inflammatory cell infiltration was found, which consisted of macrophages, neutrophils and T cells ($p<0.01$). SEM of corneal surface after exposure termination showed time-dependent recovery in the intercellular gaps and epithelial cell sloughing, which previously increased during exposure. While our previous study found increased tear film concentrations of IL-8, IL-17 and TNF-α between 0h to 4h of exposure termination, these cytokines showed recovery at 24h with no significant difference from before 24h of exposure termination ($p>0.05$). There was no change in IL-1 and IL-2 concentrations during the entire observational period ($p>0.05$).
Conclusions: Recovery of inflammatory response and ocular surface structure takes time after recovery of exposure keratopathy. Time-dependent decrease in several cytokines was found in tears within 24h after termination of eye exposure.
Commercial Relationships: Wei-Li Chen, None; **Lily Chen**, None; **Wen-Hui Tu**, None; **Fung-Rong Hu**, None
Support: 103-2314-B-002-074-MY3

Program Number: 491 **Poster Board Number:** A0416
Presentation Time: 1:30 PM–3:15 PM
Variation of the leukocyte composition in the open eye of normal and dry eye subjects
Cameron K. Postnikoff[2], Carrie E. Huisingh[1], Gerald McGwin[1], Kelly K. Nichols[2]. [1]Ophthalmology, University of Alabama at Birmingham, Birmingham, AL; [2]School of Optometry, University of Alabama at Birmingham, Birmingham, AL.
Purpose: The closed eye is characterized by a large neutrophil influx, but little is known about the presence or absence of leukocytes in the awake, open eye tear film. This study sought to compare normal subjects and dry eye subjects for daily variation in open eye leukocyte composition.
Methods: Nine normals and six dry eye subjects were enrolled, with a combined average age of 32 years, and subjects were 70% female. Dry eye subjects were recruited based upon a prior diagnosis of dry eye disease, and were not taking any ocular medications. Subjects were trained for self-collection of leukocytes using a gentle wash of the ocular surface, using 5 mL of phosphate buffered saline per eye. Subjects collected tears at four different time points across four separate days: at awakening, between 8-9am, between 11am-12pm, and between 4-5pm. Tear samples were processed and leukocytes were counted using a Moxi Z cell counter. Samples were then stained with CD45 and a fixable viability stain to confirm the presence of neutrophils or lymphocytes based on CD45+ staining and forward scatter/side scatter sample characteristics. General linear models using generalized estimating equations were used.
Results: Subjects had been awake for 2.5±0.9, 5.2±1.22, and 9.9±0.99 hours, at the 8am, 11am, and 4pm collections, respectively. Overall, roughly 813,000 cells were collected after a full night of sleep. At 8am, 11am, and 4pm, the total average number of recovered cells from normals was 6,266, 14,142, and 14,048, and dry eye subjects was 5,913, 8,558, and 11,914, respectively, as measured by the Moxi Z cell counter. Overall, there were significantly fewer leukocytes in the open eye versus the closed eye (at awakening) (p<0.001), but there was no difference between the open eye time points (p=0.21). There was no statistically significant difference between normals and dry eye subjects in terms of leukocyte recovery in the open eye (p=0.83). Flow cytometry demonstrated the presence of both neutrophils and lymphocytes, but yields were too low for phenotypic analysis.
Conclusions: Within two and a half hours of eye opening, closed eye leukocytes are rapidly cleared from the ocular surface. However, the open eye tear film does contain a tonic level of both neutrophils and lymphocytes, both in normal and dry eye subjects, which may play a role in ocular surface homeostasis.
Commercial Relationships: Cameron K. Postnikoff, None; **Carrie E. Huisingh**, None; **Gerald McGwin**, None; **Kelly K. Nichols**, None
Support: AAO Section on Cornea, Contact Lenses, and Refractive Technologies Ezell Fellowship, NSERC PGSD3 Scholarship

Program Number: 492 **Poster Board Number:** A0417
Presentation Time: 1:30 PM–3:15 PM
Osmolarity-dependently Protective Effects of Trehalose on Inflammatory Markers in Primary Human Corneal Epithelial Cells Exposed to Hyperosmotic Stress
Zhao Liu[1, 2], XIN CHEN[1, 3], DING CHEN[1, 3], Stephen C. Pflugfelder[1], De-Quan Li[1]. [1]Ocular Surface Center, Cullen Eye Institute, Department of Ophthalmology, Baylor College of Medicine, Houston, TX; [2]Department of Ophthalmology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China; [3]School of Ophthalmology & Optometry, Wenzhou Medical University, Wenzhou, China.
Purpose: Hyperosmolarity has been recognized as a pro-inflammatory stress in the pathogenesis of dry eye disease. This study was to explore the protective effects of the disaccharide trehalose on expression and production of pro-inflammatory mediators in primary human corneal epithelial cells (HCECs) exposed to hyperosmotic stress.
Methods: Primary HCECs were established from fresh donor limbal tissue explants. The cultures in iso-osmolar medium (312 mOsM) were switched to hyperosmotic media (450 mOsM) by adding 70 mM NaCl, with or without prior incubation of different concentrations (0.1-5%) of trehalose, alone or in combination of another osmoprotectant, L-carnitine, for different times (4-24 hours). The mRNA expression by HCECs was determined by reverse transcription and quantitative real time PCR. The protein production in the conditioned media from cultures was evaluated by ELISA.
Results: The expression of pro-inflammatory cytokines, TNF-α, IL-1β and IL-6, and chemokine IL-8 was significantly stimulated in HCECs exposed to hyperosmotic medium (450 mOsM). This stimulation was largely suppressed at both mRNA and protein levels by prior-treatment with trehalose at 0.5-1.5% while 1.0% showed the best effect. Higher concentrations (2.0-3.0%) of trehalose reduced the suppressive effects on these pro-inflammatory markers in a concentration-dependent manner, and 5% had no suppressive effect, or was associated with higher expression of these markers. This phenomenon was likely due to the higher osmolarity (500~570 mOsM) generated by 2.0-5.0% of trehalose in the HCEC hyperosmolarity model. Prior treatment with 1.0% trehalose in combination with L-carnitine at 10 mM significantly enhanced the inhibitory effects on these pro-inflammatory markers at mRNA and protein levels, causing 20-50% further down-regulation. Further investigations are going on to explore the differential molecular mechanisms initiated by trehalose and L-carnitine.
Conclusions: Our findings demonstrate that hyperosmotic stress stimulates the expression and production of proinflammatory mediators in HCECs. Trehalose functions as an osmoprotectant, producing an osmolarity-dependent suppression of inflammatory responses. L-carnitine further enhances the protective effects initiated by trehalose, suggesting that the 2 compounds may act by different mechanisms and have synergistic effects.
Commercial Relationships: Zhao Liu, None; **XIN CHEN**, None; **DING CHEN**, None; **Stephen C. Pflugfelder**, Allergan, plc (F); **De-Quan Li**, Allergan, plc (F)
Support: NIH NEI Grants R01EY023598 (DQL), EY011915 (SCP) and Core Grant for Vision Research EY002520, Research to Prevent Blindness, Oshman Foundation, William Stamps Farish Fund, and Allergan, plc.

Program Number: 493 **Poster Board Number:** A0418
Presentation Time: 1:30 PM–3:15 PM
Scientific Considerations for In Vitro Bioequivalence Studies of Generic Cyclosporine Ophthalmic Emulsions
Darby Kozak[1], Mohammad Absar[1], Peter Petrochenko[1], Xiaoming Xu[3], Jiwen Zheng[2], Yong Wu[2]. [1]Office of Generic Drugs, US Food and Drug Administration, Silver Spring, MD; [2]Office of Science and Engineering Laboratories, US Food and Drug Administration, Silver Spring, MD; [3]Office of Pharmaceutical Quality, US Food and Drug Administration, Silver Spring, MD.
Purpose: Currently, the FDA recommends two options to demonstrate bioequivalence (BE) for cyclosporine ophthalmic emulsion (COE): (1) a clinical endpoint study or (2) in vitro studies with comparative physicochemical characterization. As the in vitro option relies on demonstrating product sameness, the FDA requires generics approved by this option be formulated qualitatively and quantitatively similar to the reference product. A summary of scientific considerations when conducting studies to support the in vitro option was compiled and certain complex parameters such as particle size and viscosity were evaluated.
Methods: For development of the in vitro option, a systematic approach was adopted to identify the physicochemical parameters that are critical to the COE formulation properties as well as considerations of analytical and compartive methods. Particle size distribution (PSD) at different dilutions was measured using dynamic light scattering, laser diffraction and transmission electron microscopy (TEM). Both negative staining TEM and cryo-TEM – was used to measure globule size and morphology of the formulation. A full viscosity profile as a function of applied shear was measured using a cone-and-plate geometry.
Results: In addition to product manufacturing conditions, analytical procedures can impact the measured physicochemical properties of COE. COE appears to have a polydisperse/multimodal PSD, which can range from a few nanometers up to a micron depending on the instrument and sample preparation. As such complementary analytical sizing methods are recommended as no one single method may be suitable to measure the entire size range. In addition, more complex histogram analysis, such as earth mover's distance, is suggested for comparing PSD sameness. Finally, the COE formulation is non-Newtonian shear-thinning; therefore, viscosity profiles as a function of applied shear rate are recommended.
Conclusions: The BE guidance on COE provides further essential details on the in vitro study parameters and evaluation criteria. Due to the polydisperse nature of COE (ex. oil globules, micelles) and presence of a carbomer copolymer excipient, special considerations must be employed for particle size and viscosity measurements as well as in the method for comparative analysis to ensure generic and reference product sameness.
Commercial Relationships: Darby Kozak, None; **Mohammad Absar**, None; **Peter Petrochenko**, None; **Xiaoming Xu**, None; **Jiwen Zheng**, None; **Yong Wu**, None

Program Number: 494 **Poster Board Number:** A0419
Presentation Time: 1:30 PM–3:15 PM
A Galectin-3-Based Slot Blot Affinity Assay for MUC16
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Purpose: MUC16 and galectin-3 contribute to the formation of the ocular surface glycocalyx barrier. The ability to examine the affinity between MUC16 and galectin-3 could aid in understanding the impact of ocular surface disease on the integrity of the glycocalyx. The purpose of this work was to adapt a slot blot assay to determine the relative affinity of galectin-3 for MUC16 collected from human tears.
Methods: Tear film samples of up to 15 µl were collected from each eye of 14 normal subjects and both samples were pooled for each subject. Total protein was determined for each pooled sample using the MicroBCA assay. Nitrocellulose membranes on a 48-well Bio-Dot slot microfiltration unit were loaded with 500 ng of recombinant human galectin-3 (rhGal-3), vacuum filtered, and incubated with 5, 10, and 15 µg of tear protein. One well was loaded with 5 µg of tear protein from each subject without rhGal-3 as a control. MUC16 binding was detected using the M11 monoclonal antibody by chemiluminescence and quantified by densitometry. Levels of complex N-linked oligosaccharides in tears were determined by lectin blot using *Phaseolus vulgaris* agglutinin (PHA-L). The Kruskal-Wallis test was performed to compare the relative amounts of MUC16 bound to rhGal-3.
Results: The average total protein concentration obtained from each pooled sample was 2.26 ± 0.99 µg/µl (range 1.26 - 5.16 µg/µl). By slot blot, the average densitometry value for MUC16 in control samples was 6145.18 ± 2953.63 (range 1574.36 – 11901.52). The median values for the normalized relative amount of MUC16 bound to rhGal-3 for 5, 10, and 15 µg of protein were 0.67, 0.86, and 1.02. Statistical analysis to compare the relative amount of MUC16 bound to rhGal-3 with each amount of tear protein approached statistical significance ($H = 5.09$, $p = 0.08$). Lectin blot with PHA-L revealed the presence of two distinct bands with molecular weights of approximately 250 kDa in the tear fluid.
Conclusions: Slot blot is a viable method to determine the relative binding affinity of MUC16 to rhGal-3 using tear samples.
Commercial Relationships: Anna F. Ablamowicz, None; **Ashley Woodward**, None; **Jason J. Nichols**, None; **Pablo Argueso**, None
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Presentation Time: 1:30 PM–3:15 PM
An Evaluation of Cosmetic Wear Habits Correlated to Ocular Surface Disease Symptoms
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Purpose: There has been very little investigation about the relationship between cosmetics and ocular surface disease. This study looked at application and removal habits of cosmetic wearers and non-wearers to determine any relationship that cosmetics may have on ocular surface discomfort and symptomology.
Methods: A survey was created and distributed using the media platform 'Survey Monkey' and social media outlets including Twitter, Facebook, LinkedIn and email. Data from the survey was compiled and evaluated in order to identify any relationships between cosmetic wear habits, ocular health history and ocular symptoms.
Results: A total of 253 people responded to the survey, with a mean age of 41.67 + 10.04. Those surveyed reported wearing makeup an average of 4.99 + 2.22 days per week. Additionally reported, make-up was removed an average of 4.68 + 2.49 days. Of those surveyed, the average SPEED score was 8.19 + 5.18 and the average UNC = 3.28 +2.32. Those that did not report using an eye make up remover had higher SPEED scores (Mean:10.5 +6.75) than those

who did use eye make up removers (Mean 7.6+4.62) P=0.0004. Data analysis also revealed that contact lens wearers noted more vision fluctuations after makeup removal than those who are not contact lens wearers (p=0.000429).
Conclusions: Consumers and patients are using eye cosmetics frequently. Eye Care Providers need to be aware of their patients' habits for application and removal and consider both as potential for ocular discomfort. More research is needed to explore the relationship between Ocular Surface Disease and cosmetic use.
Commercial Relationships: Leslie E. O'Dell, None; **Laura M. Periman**, None; **Amy G. Sullivan**, None; **Clare Halleran**, None; **Jennifer S. Harthan**, None; **Milton M. Hom**, None

Program Number: 496 **Poster Board Number:** A0421
Presentation Time: 1:30 PM–3:15 PM
DRY EYE DISEASE PREVALENCE IN EMPLOYERS OF TWO HEALTH SERVICE INSTITUTIONS IN BOGOTA COLOMBIA
Sergio A. Arrascue[1], Andrés F. Polit[1], Juanita Carvajal[1], Sandra Talero[2], Clara Lopez[1]. [1]Escuela Superior de Oftalmología, Bogota, Colombia; [2]Ophthalmology, Hospital Universitario de La Samaritana, Bogota, Colombia.
Purpose: To find the prevalence of dry eye disease in employers of two Health service institutions
Methods: Transversal descriptive study of 307 patients from two diferent Health service institutions in Bogota D.C. The Barraquer Clinic and the Samaritana University Hospital, between June and december of 2015. The participants had inform consent for the Schimer and BUT test. Symptoms and signs were registered during the ophthalmoscopic exam.
Results: The average age of the patients was 40 +/- 12 años (R: 18 – 65), 73.3% were females and 26.7% males.
The prevalence of dry eye was 64.2% (n = 197), in the Samaritan Hospital was 56.3% and in Barraquer Clinic was 71.8% (p = 0.005). The presence of dry eye had a higher proportion in participants under 29 years (32%), the prevalence in woman was 67.1% and in men was 56.1% (p=0.07). The 54.9% of participants with dry eye had demodicosis Vs no demodicosis in 41.5% (p=0.006) and a association with blepharitis in 60.2% Vs no blepharitis in 39.8% (p=0.025). According to the work position of de employees la frequency of dry eye was in analysts (87.5%), secretaries (76.5%), auxiliarys (64.2%), health workers (62.1%) and general workers (50%).
In relation to the symptoms, the most relevant symptoms were erythema in the palpebral borders: dry eye (61.1%) vs no dry eye (38.9%) (p=0.006). Telangiectasis in cheecks or nose: dry eye (61.3%) Vs no dry eye (38.9%) (p=0.025). Ocular hyperemia: Dry eye (48.9%) Vs no dry eye (51.1%) (p=0.03).
Conclusions: The two institutions had a high prevalence of dry eye, specially in woman and in patients under 30 years. A high frequency of dry eye asociated with blepharitis and demodicosis. The employees with a high prevalence of dry eye disease were analyst.
Commercial Relationships: Sergio A. Arrascue, None; **Andrés F. Polit**, None; **Juanita Carvajal**, None; **Sandra Talero**, None; **Clara Lopez**, None
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Program Number: 497 **Poster Board Number:** A0422
Presentation Time: 1:30 PM–3:15 PM
Effect of proinflammatory cytokines on expression of corneal and conjunctival epithelial mucins: Possible implications for GVHD associated dry eye
Ajay Sharma[2], Sally Sun[1], Jonathan Taniguchi[1], Sumit Garg[2], Marjan Farid[2]. [1]Chapman University School of Pharmacy, Irvine, CA; [2]Gavin Herbert Eye Institute, University of California, Irvine, CA.
Purpose: Dry eye is a frequent and serious complication of graft versus host disease (GVHD). Levels of a variety of proinflammatory cytokines are significantly elevated in tears of GVHD patients. Mucins are glycosylated proteins that are crucial for ocular surface hydration. Thus, a decrease in mucins expression can have a significant negative impact on tear film. The present study investigates the effect of proinflammatory cytokines TNFα and IL-6 on expression of ocular mucins.
Methods: Human conjunctival and corneal epithelial cells were used. Conjunctival cells were grown in serum-free low calcium F12/DMEM medium, and then switched to serum-containing keratinocyte medium for stratification. Corneal epithelial cells were grown on transwell membrane inserts, in growth factor-supplemented complete keratinocyte medium. The cells were exposed to different doses of TNFα and IL-6 for 24 hours. The cells were harvested for mRNA isolation and preparation of protein lysates. The mucins 1, 4, 5AC, 16 &19 gene expression and protein quantification was done using real time PCR and ELISA respectively. Statistical analysis was performed by one-way ANOVA and tuckey's test.
Results: Both TNFα and IL-6 caused a decrease in the gene and protein expression of mucins in corneal and conjunctival cells. The decrease was dose dependent. The doses that recapitulated the published values of tear levels in the GVHD patients caused the most significant decrease. Our results tentatively suggest that elevated levels of proinflammatory cytokines may contribute to the pathogenesis of dry eye by decreasing the expression of ocular mucins in GVHD.
Conclusions: Based on our results it may be concluded that proinflammatory cytokines such as TNFα and IL-6 decrease the expression of mucins in the corneal and conjunctival epithelial cells. Since the levels of these cytokines are significantly elevated in the tear fluid of GVHD patients, cytokine-mediated decrease in mucins can significantly contribute to the pathogenesis of GVHD associated dry eye.
Commercial Relationships: Ajay Sharma, None; **Sally Sun**, None; **Jonathan Taniguchi**, None; **Sumit Garg**, None; **Marjan Farid**, None

Program Number: 498 **Poster Board Number:** A0423
Presentation Time: 1:30 PM–3:15 PM
Reduction in inflammatory marker matrix metalloproteinase-9 following lid debridement with BlephEx
Charles G. Connor, Srihari Narayanan, William Miller. Rosenberg School of Optometry, University of the Incarnate Word, San Antonio, TX.
Purpose: Meibomian Gland Disease (MGD) is a common cause of evaporative dry eye. Lid scrubs and warm compresses can address this problem but poor compliance makes an office based procedure desirable. Korb found the debridement-scaling of the lower lid margin provides statistically significant symptom relief and improvement in MG function. The BlephEx provides a method of accomplishing lid debridement without using a surgical instrument. Ocular surface inflammation is well documented in dry eye patients. The RPS InflammaDry recognizes elevated levels of MMP-9, an

inflammatory marker that is consistently elevated in the tears of dry eye patients. The purpose of this study is to determine if MMP-9 levels change following lid debridement with BlephEx.
Methods: Ten MGD patients (6 male, 4 female) with evaporative dry eye (Age range 25 to 57; mean age 30.25+/- 10.85) who tested positive on the RPS InflammaDry participated in this study. The OSDI, NITBUT and Meibography grading were performed just prior to BlephEx treatment. All tests were repeated four weeks post-BlephEx treatment. The RPS Inflammadry test was also performed pre-and post-BlephEx treatment. Data was analyzed by a t-test with post hoc test for significance.
Results: All patients had a significant ($p<0.01$) reduction of symptoms four weeks after BlephEx treatment with baseline OSDI score reducing from 26 +/- 13.37 to 10.66 +/- 7.09. The NITBUT improved significantly ($p<0.05$) from a baseline of 6.99 +/- 2.37 seconds to 9.53 +/-2.41 seconds post-treatment. Meibography grades (Pult grading scheme; Oculus 5M Keratograph) did not change significantly (pre-treatment Grade 1.5 to grade 1.375 post-treatment). Most importantly, all ten subjects were negative for MMP-9 using the Inflammadry test at four weeks post-BlephEx treatment.
Conclusions: Subjects were over 50% less symptomatic and had a better NITBUT following BlephEx treatment. MMP-9 marker was negative for all subjects at four weeks post-BlephEx treatment. The betterment in symptoms can possibly be explained by the reduction in ocular surface inflammation. The results of this study suggest that debridement of the lid margin offers subjective and objective benefits to MGD patients.
Commercial Relationships: Charles G. Connor, None; **Srihari Narayanan**, None; **William Miller**, None

Program Number: 499 **Poster Board Number:** A0424
Presentation Time: 1:30 PM–3:15 PM
Trehalose/Hyaluronate eyedrops effects on ocular surface parameters, inflammatory markers and mucin expression in evaporative Dry Eye patients
Emilio C. Campos, Piera Versura, Giuseppe Giannaccare, Michela Fresina, Chiara Fariselli. DIMES, Ophthalmology Unit, Alma Mater Studiorum University of Bologna, Bologna, Italy.
Purpose: To assess the levels of ocular surface functional parameters, inflammatory markers in tears and mucin expression in conjunctival epithelium before and after topical treatment with Trehalose/Hyaluronate based tear substitute (Thealoz® Duo, laboratoires Théa, France) in dry eye (DE) patients
Methods: Fifteen evaporative DE patients (fourteen women aged 54 yrs as a median, one man aged 58 yrs, DEWS severity level 2-3) were evaluated in an open label, not comparative, pilot study at enrollment (V0), after 2 days of washout (baseline, V1), and after 1 month (V2) and 2 months (V3, endpoint) of treatment with Thealoz® Duo (one drop/eye/4 times daily). Data for ocular subjective symptoms of discomfort (OSDI and VAS pain score), tear film (Schirmer test I, TFBUT), ocular surface damage (corneal NEI and conjunctival van Bijsterveld scores, impression cytology scored by Nelson's grade and goblet cells (GC) number/mm^2 analysis, and MUC4 immuno-staining evaluated by H-score) and inflammation (IL-1β, IL-6 and IL-8 levels) were measured. The association among different variables were also calculated
Results: Parameters showing significant (always $p<0.01$) changes at endpoint as compared to baseline were: OSDI score (respectively mean±SD, 22.2±2.9 vs 38.7±12.7), VAS score (3.4±1.3 vs 6.6±1.4), TFBUT (7.5±1.9 vs 5.4±2.3 sec), corneal staining (NEI grade 1.23±0.64 vs 3.37±0.49), conjunctival staining (1.73±0.64 vs 4.17±0.91), impression cytology (Nelson grade 1.10±0.20 vs 1.63±0.54), GC density (139.9±22.0 vs 107.8±16.2 GC/mm^2). IL-1b, IL-6 and IL-8 tear levels were found above the minimum detectable level in all subjects at baseline (respectively mean±SD, pg/ml tears: 33.6±17.3, 112.0±24.3 and 1139.2±671.7) with a significant decrease at endpoint (12.3±6.9, 26.6±25.2 and 743.5±477.7), $p<0.01$. Cytokine levels were correlated to surface damage at baseline (Pearson's r ranging 0.45-0.59, $p<0.0001$) but not at endpoint
Conclusions: A decrease in ocular discomfort symptoms, surface damage and tear cytokine levels was shown after two month treatment with Thealoz® Duo eyedrops in DE patients, along with a significant GC density recovery. These results may be associated to the synergic action of both trehalose and hyaluronic acid in targeting different entries of the DE vicious loop
Commercial Relationships: Emilio C. Campos, None; **Piera Versura**, None; **Giuseppe Giannaccare**, None; **Michela Fresina**, None; **Chiara Fariselli**, None