3361 - D947

Parametric vs. Non-Parametric Analysis to Assess Ocular Protection Index (OPI) in Cross-Over Designs

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Purpose: Parametric and non-parametric methods were applied to OPI data from a three-visit, three-treatment ophthalmic cross-over study to determine which method is better at identifying statistically significant differences. The OPI response variable is the primary endpoint and it is often positively skewed. Traditionally, parametric analysis methods are requested to be used for the primary analysis. It is clear that the parametric model assumptions (i.e., normally distributed data) are not entirely valid when analyzing OPI data. Therefore, other more appropriate analysis methods (e.g., non-parametric) can potentially improve the ability to identify statistically significant differences.
Methods: The results from the study were originally analyzed using two parametric methods: a repeated measures analysis was performed adjusting period and sequence using a mixed model, and a two sample t-test making pairwise comparisons at each of the individual visits. Two non-parametric analyses were run post-hoc: an overall non-parametric analysis adjusting for period, and a basic non-parametric analysis with Hodges-Lehmann type estimator. Resultant P-values were compared to determine if the non-parametric methods perform equivalently or better than the parametric.
Results: Pairwise comparisons were performed among two active treatments, Drug C and Drug K, and one placebo. For both the per protocol (PP) and intent-to-treat (ITT) populations, more statistically significant differences resulted when using the non-parametric methods. Furthermore, both non-parametric methods produced very similar results.
Conclusions: Non-parametric methods, when applied to OPI data in cross-over designs, have a greater ability to identify statistically significant pairwise comparisons when compared to parametric methods. These methods do not require any distributional assumptions. Therefore, model assumption validity is relaxed. Furthermore, both non-parametric methods produced consistent results.
CR: D. Kennedy, Statistics and Data Corporation, E; **Y. Li**, Statistics and Data Corporation, E; **K. Kennedy**, Statistics and Data Corporation, E; **H.-C. Hsu**, Statistics and Data Corporation, E; **D. Jethwani**, Statistics and Data Corporation, E.
Support: None

3362 - D948

Enhanced Precision of the Controlled Adverse Environment (CAE) System

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Purpose: Successful study of dry eye therapies requires the use of standardized clinical models that generate reproducible results. Researchers at Ora, Inc developed the enhanced CAE system due to the evolving knowledge of dry eye subgroups, evolution of clinical grading scales, inclusion and exclusion criteria and time points. The purpose of this research was to evaluate the variability of clinical findings using the enhanced CAE system.
Methods: The variability of signs and symptoms were assessed using data on file from studies using the enhanced CAE system and historical data from studies using the classic CAE. The enhanced CAE system incorporated advances in the technology and design, allowing for evaluation during CAE exposure, during recovery periods after CAE exposure, and during environmental exposure between CAE exposures.
Results: The ranges of standard deviations (SD) for ocular discomfort and corneal staining as measured using the enhanced CAE system were substantially lower than those recorded using the classic CAE.
Conclusions: The results of the present research demonstrate the increased precision in the signs and symptoms of dry eye when assessed using the enhanced CAE system. The data illuminate the successful evolution of the CAE trial design by incorporating substantial technological and design improvements in the enhanced CAE system. Ongoing research using the enhanced CAE system may confer advanced identification of disease sub-groups and improved precision in clinical endpoints, as well as continuing to promote pharmacological success in the future.

	Ocular Discomfort during CAE exposure (Range of SD)	Pre-CAE Corneal Fluorescein Staining* (Range of SD)	Post-CAE Corneal Fluorescein Staining* (Range of SD)
Classic CAE (N = 112)	0.74 – 1.46	0.67 – 0.72	0.86 – 0.95
Enhanced CAE system (N = 248)	0.460 – 0.985	0.531 – 0.591	0.593 – 0.602

**Fluorescein staining SDs are listed for the inferior corneal region*

CR: G.W. Ousler, III, Ora, Inc., E; **M.B. Abelson**, Ora, Inc., E; Schepens Eye Research Institute and Harvard Medical School, E; **D.L. Welch**, Ora, Inc., E.
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3363 - D949

Clinical Predictors of Sjögren's Syndrome Diagnosis in a Population of Individuals With Dry Eye

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Purpose: To determine whether medical history, ocular exam characteristics, and serologic test results of individuals who present with symptoms of dry eye can be used to predict eventual diagnosis of Sjögren's Syndrome (SS).
Methods: Medical records of patients with a primary diagnosis of dry eye syndrome (International Classification of Diseases [ICD] code 375.15, 370.33 and 710.2) were reviewed retrospectively. These individuals had presented to the Dry Eye clinic between January 2002 and May 2009. Among these individuals, those who underwent additional serologic work-up for SS were selected.
Results: Among 1538 individuals who were referred with symptoms of dry eye syndrome, as determined by schirmer levels, 237 underwent additional serologic work-up for suspected SS and were selected for further analysis. Among these 237 individuals, 52.7% tested positive for ANA and 21.6% for rheumatoid factor, 8.3% for SSA antibodies and 7.4% for SSB antibodies. 27% were seronegative and underwent minor salivary gland biopsy and of these, 48.5% had biopsy grade 3 or above. 89 (37.6%) of these individuals were eventually diagnosed with SS based on the European-American criteria. In a univariate analysis, gender, age, duration of symptoms, and presence of other co-morbid rheumatologic conditions (P> 0.05) did not differ significantly between the SS and non-SS groups. Features significantly associated with an eventual SS diagnosis included presence of dry mouth (P=0.015), Raynauds (P = 0.023), corneal staining (P=0.003), family history of SLE (P = 0.044), DM Type I (P = 0.033), SSA (OR = 40.2), SSB (OR = 35.4), and RF (OR = 5.6). ANA correlated in a dose responsive fashion with higher titers associated with an SS diagnosis (titer 1:640, OR = 7.4). A positive biopsy (P<0.000) was also strongly associated with the eventual diagnosis.
Conclusions: Primary SS is a common cause of dry eye and should be the focus of diagnostic evaluations. The study identifies additional factors on review of systems, history, ocular exam and serology to identify individuals who have higher odds of being diagnosed with Sjögren's Syndrome. The results of the study can be used as a guide to select for individuals who should undergo additional serologic testing to confirm diagnosis and to initiate treatment.
CR: R.N. Swamy, None; **C.A. Utine**, None; **J.E. Thorne**, None; **A. Baer**, None; **E.K. Akpek**, None.
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3364 - D950

Predicting Post-Operative Dry Eye: Multivariate Analysis of Clinical Findings, Tear Proteins, and Goblet Cells

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Purpose: To examine the significance of subjective symptoms, the clinical examination, conjunctival goblet cells, and inflammatory tear proteins and cytokines in predicting post-operative dry eye in refractive surgery patients.
Methods: A prospective, non-randomized, multicenter study comparing the effect of LASIK and PRK on the clinical findings of cochet-bonnet aesthesiometry, tear break up time (TBUT), rose Bengal staining (RB), videokeratoscopy surface indices, percent of filled goblet cells (GC), 16 cytokines, 7 matrix metalloproteinases, 2 matrix metalloproteinases inhibitors, and subjective sxs of dry eye quantified using the McMonnies questionnaire. Discriminant analysis was conducted at 1 week (1W), 1 month (1M), and 3 months (3M) to determine if significant differences existed across the predictor variables of two groups: subjects with and without postoperative dry eye.
Results: Seventy-two eyes underwent either PRK(n=39) or intralase LASIK (n=33). At one week post-op, only MMP10 (Wilks' lambda=0.461) and IL1α(Wilks' lambda=0.302) were significant predictor variables. At one month, percent filled of goblet cells (WL=.791), Rose Bengal Staining (WL=0.617), and age (WL=0.533) were different across the two groups. At 3M, McMonnies questionnaire (WL=0.724), Rose Bengal staining (WL=0.565), Schirmer's with anesthesia (WL=0.478), and age (WL=0.413) were significantly different across the two groups. The level of tear proteins were not significantly different across the two groups at either 1M or 3M and at no time point did videokeratoscopy surface indices distinguish between the dry eye group and non-dry eye group.
Conclusions: In the immediate post-operative period, there are changes in both the magnitude of tear proteins and goblet cells that contribute to dry eye. Beginning at 1M, the clinical exam and patient demographics are the best predictors of post-operative dry eye
CR: C.D. Coe, None; **K.S. Bower**, None; **D.S. Ryan**, None; **R.A. Sack**, Suny Research Foundation, F; **P. Pavel Iserovich**, None; **M.A. Shatos**, None; **D.A. Dartt**, None.
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3365 - D951
Ocular Surface Thermographer: A New Device for Dry Eye Screening

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Purpose: To evaluate the reliability of a newly developed, ocular surface-oriented thermography for dry eye screening.
Methods: Ocular surface temperature was measured in 30 eyes of 30 dry eye patients (D group, mean±SD, 52.9±17.1 yrs) and 30 eyes of 30 normal subjects (N group, 42.7±17.0 yrs) using an ocular surface-oriented, infrared radiation thermographic device in a non-contact manner (Ocular Surface Thermographer; OST, TOMEY Co). Subjects were asked to keep their eyes open continuously for 10 seconds after closing them for 5 seconds. The temperatures of three regions, including the center of the cornea, and the nasal and temporal conjunctiva, were determined immediately after eye opening, and after 10 seconds of sustained eye opening. In addition, correlations between changes in surface temperature and tear film break-up time (BUT), Schirmer I test values, and corneal and conjunctival epithelial fluorescein staining scores were analyzed.
Results: Immediately after eye opening, no significant difference was noted between the surface temperatures of the N and D groups in any of the 3 regions, while the temperature of the D group was found significantly decreased as compared with that of the N group after 10 seconds of sustained eye opening (center of the cornea $p < 0.001$, nasal conjunctiva $p < 0.05$, temporal conjunctiva $p < 0.01$). The decrease in temperature at the center of the cornea was particularly noteworthy, and was significantly correlated with BUT ($r = -0.572$, $p < 0.001$). When the change in temperature of the center of the cornea (> 0.13 °C as a cut off value) was applied as an index for dry eye screening, the sensitivity and specificity for 10 seconds of sustained eye opening were 0.83 and 0.80, respectively.
Conclusions: Measurement of ocular surface temperature during sustained eye opening using OST may be useful for simple dry eye screening.
CR: M. Yamaguchi, None; **T. Kamao**, None; **S. Kawasaki**, None; **S. Mizoue**, None; **A. Shiraishi**, None; **H. Kataoka**, TOMEY Corporation, P; **Y. Ohashi**, None.
Support: None

3366 - D952
Blink Patterns in Normal and Dry Eye Subjects; Beyond Blink Rate

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Purpose: Blink rate has long been studied in conjunction with dry eye and it has been shown that non-dry eye subjects blink significantly less than subjects diagnosed with dry eye while watching TV, using a computer and reading[1]. The current study evaluated not blink rate alone, but blink patterns, meaning the occurrence of blink types, (extended, full, partial), varying interblink intervals, and the completeness of blinks over time. This study was conducted to better characterize the presentation of blink patterns in normal and dry eye subjects. [1] P Walker, R White K Lane, MB Abelson. A Comparative Assessment of Blink Rates across a Series of Tasks in Dry Eye and Control Patients. ARVO 2007 5317/D759..
Methods: A total of 20 subjects participated (n=10 diagnosed with dry eye, n=10 normal). Blink patterns were collected over a ten minute period while the subject performed a controlled visual task (television watching) and were compared between populations.
Results: Dry eye subjects and non dry eye subjects had average IBIs of 2.61 and 5.57 seconds, respectively ($p=0.002$). Maximum IBI was significantly different for the two groups, 14.34 and 25.19 seconds respectively ($p=0.034$). Variance of IBI also differed significantly between the groups and was shown to be higher in normals than dry eye (0.28 difference in standard deviation between populations, $p=.004$)) The two populations were significantly different in incidence of extended full blinks, occurring 1% and 4% of the time in dry eye and non dry eye subjects, respectively ($p=0.031$).
Conclusions: We have shown that these two patient populations are clearly differentiated in blink pattern and have confirmed increased frequency of blinks in dry eye subjects as well as the inability of dry eye subjects to sustain extended IBIs. This differentiation between the two groups may provide a better understanding of the role of blink patterns in dry eye and their potential modulation in therapeutic trials.
CR: R. White, Ora Inc, E; **J. Rodriguez**, Ora Inc, E; **K.J. Lane**, Ora Inc., E; **P. Johnston**, Ora Inc., E; **E. Angjeli**, Ora Inc., E; **M.B. Abelson**, Ora Inc., E.
Support: None

3367 - D953
Analysis of Blink Parameters and Tear Film Instability With Tasks

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Purpose: While it is recognized that the purpose of the blink is to lubricate the corneal surface, few have studied the relationship between blinking and the tear film. This study investigated how the parameters of the blink changed with concentration (internal controls) and surface anesthetic (external controls) and whether the blink adequately rewetted the corneal surface.
Methods: Ten subjects (2 males and 8 females) completed the Dry Eye Questionnaire (DEQ). A small reflective dot was placed on one eye and 2μl of 2% sodium fluorescein was instilled. Tear film stability and blinking (via tracking of dot) were monitored by 2 digital cameras while listening to music and playing a computer game, with and without anesthetic. Blink rate (BR) and amplitude (BA), and up (UPV) and down phase velocity (DPV) and the area of tear break-up (TBU) were analyzed using custom designed MATLAB programs.
Results: BR (AVG/min ±Std) was significantly slowed (paired t-test, $p<0.02$) during the game (8.87±6.06 without and 5.63±5.75 with anesthetic) compared to music (15.12±10.43 without and 14.3±10.57 with anesthetic). Other blink parameters (%BA, UPV%/sec, DPV%/sec) were the following: game with (59.08/2.31/7.08) and without anesthetic (66.84/2.44/7.93); music with (66.03/1.95/6.26) and without (67.98/1.9/6.83) anesthetic. Both UPV and DPV were significantly increased with the game compared to music and the DPV was significantly decreased with anesthetic (t-test, $p<0.01$). The AVG TBU% (before/after each blink) was significantly greater during the game, both with (25.31/14.15) and without (15.17/10.66) anesthetic, compared to music with (17.96/12.29) and without (10.52/8.77) anesthetic.
Conclusions. During the game, the BR slows and UPV and DPV increase, suggesting that central/internal controls over blinking act to minimize interruption of concentration by the eyelids. Anesthetic slowed the DPV, which suggests an ocular surface/external input to blinking. Our results also indicate that many blinks are not full, especially when concentrating on a task, and often do not fully re-lubricate the corneal surface.
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3368 - D954
Tear Volume, Blink Rate, and Sensation Evoked by Humidified Air Stimulation of the Cornea in Normal Adult Humans

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Purpose: Dry eye syndrome (DES) is a disorder of inadequate tears on the ocular surface often associated with symptoms of pain, discomfort and visual disturbance. Recently, we have characterized corneal primary afferent neurons in the rat that are activated by drying and innocuous cooling, and inhibited by warming of the cornea. These neurons may be important for activating the reflex arc responsible for basal tearing and blinking. The aim of the present study was to determine whether non-noxious temperature changes of the cornea in normal adult humans affects tear production, blink rate, and subjective sensations.
Methods: The cornea was stimulated with a modified gas esthesiometer. Air was bubbled through either hot or room temperature water and delivered to the cornea at a rate set slightly below mechanical threshold for 2 minutes. Warmed humidified air was approximately 32 °C at the point of measurement immediately in front of the cornea. Tear volume was measured with phenol red threads (Zone-Quick®). Blink rate and subjective reports were determined from video recordings. Subjects were 6 male and 14 female volunteers between the ages of 18 -46. Exclusion criteria were the following conditions: DES, diabetes, history of corneal trauma, cluster headache, contact lens use and history of eye irritation.
Results: Tear volume was significantly ($p < 0.01$) reduced (X = 23.25 mm, sem= 1.87) by warm air when compared to baseline condition (no air stream: X = 26.7 mm, sem = 2.09) and room temperature air (X= 27.05 mm, sem = 2.03). Blink rate was not significantly different among the 3 experimental conditions. There was an increase in the number of subjects reporting sensations (Chi-square=11.56, df =1, $p <0.001$) between the baseline condition (4) and when stimulated (21). Subject reports did not correspond to the stimulus temperature.
Conclusions: Warming of the cornea reduces tearing. The present results support the hypothesis that corneal cold cells, which are inhibited by warming, are important for modulating basal tear production to non-noxious stimuli. Reduced activity of this class of corneal sensory neurons may contribute to DES in some individuals.
CR: B.J. Winterson, None; **A. Pasha**, None; **K. Gassman**, None; **I.D. Meng**, None.
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3369 - D955
A Novel Method for Tear Fluorescein Clearance Test Using Pentacam Scheimpflug Imaging System

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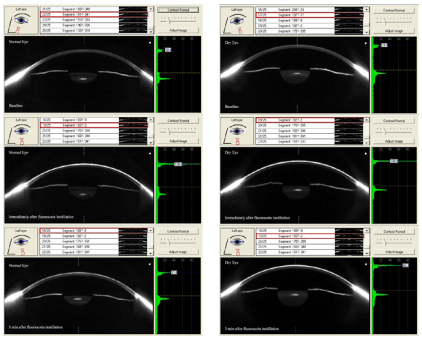
Purpose: To report a novel method for quantitative evaluation of tear fluorescein clearance (TFC) rate using Pentacam Scheimpflug imaging system.
Methods: Thirty patients with dry eye symptoms and thirty age-matched asymptomatic control subjects were enrolled in this prospective study. TFC test and SchirmerItest was performed in each participant. The TFC rate was evaluated with Pentacam by measuring reflective light intensity (RLI) of fluorescent tear film 3 minutes after instillation of 2 μl 1% sodium fluorescein into inferior cul-de-sac.
Results: Significantly decreased TFC rate (indicated by a higher tear film RLI 41.1±14.5, P < 0.0001) and lower Schirmer Score (7.0±6.4 mm/5min, P < 0.0001) were observed in symptomatic patients than those observed in normal controls (tear film RLI 17.9±13.5, Schirmer Score 18.4±9.6 mm/5min). An inverse correlation was found between tear film RLI and Schirmer Score (Spearman's rho = -0.45 , P= 0.0003). TFC test showed a slightly better value than SchirmerItest for discriminating symptomatic patients from normal controls. The cutoff value of tear film RLI 31.6 had 80 % sensitivity and 93% specificity.
Conclusions: This novel method is convenient and practical for tear fluorescein clearance test in clinic, and it will further the investigation of tear clearance under physiological and pathological conditions.



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3370 - D956
Predicting Dry Eye With Noninvasive Techniques of Tear Film Surface Assessment

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Purpose: To measure tear film surface quality (TFSQ) in healthy normal and dry eye subjects with three noninvasive techniques and to investigate their potential to predict dry eye.
Methods: Thirty four subjects, aged from 20 to 68 years were recruited for this study. Clinical assessment of the dry eye was performed using McMonnies questionnaire, FTBUT, corneal (fluorescein), and conjunctival (lissamine green) staining (NEI grading). All clinical measurements were performed by one experienced clinician who was masked with respect to the measurements performed with the noninvasive methods, which included dynamic-area high speed videokeratoscopy (HSV), dynamic wavefront sensing (DWS), and lateral shearing interferometry (LSI). The dynamic-area HSV relies on measuring changes in the recorded Placido disc pattern projected on the cornea. In DWS, temporal changes of higher order aberrations (HOA), total comatic terms (ComaV, and ComaH) and the Zernike polynomial RMS fit error were used as indicators of TFSQ. In LSI, temporal changes of the frequency characteristics of interferograms were used as a TFSQ indicator. Noninvasive measurement of TFSQ was performed in both natural and suppressed blinking conditions. Measures of TFSQ were used to calculate receiver operating characteristics (ROC) to show the capability of each technique to discriminate between dry eye and normal subjects.
Results: In the suppressed blinking conditions, the LSI method showed the best performance in terms of the area under the ROC curve, AUC=0.80, followed by HSV with AUC=0.72. The DWS achieved lower values: AUC[ComaV]=0.64, AUC[ComaH]=0.56, AUC[RMS fit]=0.57, and AUC[HOA]=0.60. In the natural blinking condition, the LSI method again showed the best performance, AUC=0.73 followed closely by HSV with AUC=0.71. Methods based on DWS showed much lower AUC values indicating poor detection performance.
Conclusions: Noninvasive techniques of tear film surface assessment can be used for predicting dry eye and this can be achieved in natural blinking as well as suppressed blinking conditions. In our study, LSI showed the best detection performance, closely followed by the dynamic-area HSV. Wavefront sensing techniques are less powerful, particularly in natural blinking conditions.
CR: D. Iskander, None; **D.H. Szczesna**, None; **D. Alonso-Caneiro**, None; **S.A. Read**, None; **M.J. Collins**, None.
Support: None

3371 - D957
Noninvasive Assesment of Tear Film Surface Kinetics

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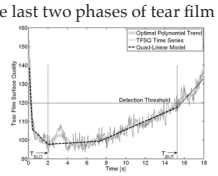
Purpose: To assess the kinetics of tear film from noninvasive measurements of tear film surface quality (TFSQ) during suppressed blinking conditions.
Methods: Twenty two normal subjects and twelve subjects with dry eye, aged from 20 to 68 years were recruited for this study. Noninvasive measurements included dynamic-area high speed videokeratoscopy (HSV), dynamic wavefront sensing (DWS), and lateral shearing interferometry (LSI). The dynamic-area HSV relies on measuring changes in the recorded Placido disc pattern projected onto the cornea. In DWS, temporal changes of higher order aberrations (HOA), total comatic terms (ComaV, and ComaH) and the Zernike polynomial RMS fit error were used as indicators of TFSQ. In LSI, temporal changes in the frequency characteristics of interferograms were used as a TFSQ indicator.
Results: A distinct tear film build-up phase was observed in over 99% of measurements in LSI, from 15% to 24% of measurements in HSV and from 24% to 50% of measurements in techniques based on DWS. The tear film stability phase, thinning phase as well as the tear film break-up (if it occurred) were observed with all instruments. Additionally, with LSI, a two-stage build-up time has been observed in both normal and dry eye subjects. The figure presents an example of estimated tear film surface kinetics for a dry eye subject using LSI. The detection threshold of break-up has been established based on statistics from measurements of TFSQ in 18 normal subjects during natural blinking. For the majority of dry eyes the tear film stability phase was shorter than in normal eyes or it was not observed.
Conclusions: The LSI technique is able to characterize up to five different phases of tear film surface kinetics that include: (1) initial fast tear film build-up phase, (2) further slower tear film build-up phase, (3) tear film stability, (4) tear film thinning, and (5), after a detected break-up, subsequent tear film deterioration. HSV as well as the DWS are suitable for characterizing the last two phases of tear film kinetics.



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3372 - D958
Noninvasive Assessment of Tear Film Surface Quality

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Purpose: To conduct a comparative study of three noninvasive techniques for assessing tear film surface characteristics of normal subjects during natural blinking conditions.
Methods: Eighteen healthy subjects were recruited for this study. A clinical assessment confirmed all subjects exhibited normal ocular surface and tear film characteristics. Dynamic-area high speed videokeratoscopy (HSV), dynamic wavefront sensing (DWS), and lateral shearing interferometry (LSI) were used to assess tear film surface quality (TFSQ). In HSV, temporal changes in the Placido disc pattern that is reflected from the tear surface were used as a TFSQ indicator. In DWS, temporal changes of higher order aberrations (HOA), total comatic terms (ComaV, and ComaH) and the Zernike polynomial RMS fit error were used as indicators of TFSQ. In LSI, temporal changes of the frequency characteristics of interferograms were used as a TFSQ indicator. Noninvasive measurement of TFSQ was performed in natural blinking conditions with the 18 subjects with all three techniques. A set of algorithms was used to derive tear film surface characteristics from each of the instruments. Parametric functions were used to fit the estimated tear film surface characteristics. Two parameters were estimated: the tear film build-up time (BLDT) and the average tear film surface quality in the stable phase of the interblink interval (TFSQ_Av).
Results: The group mean BLDT across the three techniques ranged from 0.89 to 2.46 seconds, while the group median values ranged from 0.60 to 1.92 seconds. To ascertain the precision of the instruments, the average coefficient of variation for TFSQ_Av was considered. It was less than 1% for HSV, less than 3% for LSI and ranged from 1.5% to 15% for DWS. Moderate but significant correlations were found between BLDT measured with LSI and DWS based on vertical coma (Pearson's r2=0.34, p<0.01) and higher order RMS (r2=0.31, p<0.01) as well as between TFSQ_Av measured with LSI and HSV (r2=0.35, p<0.01) and between LSI and DWS based on the RMS fit error (r2=0.40, p<0.01). No significant correlation was found between HSV and DWS.
Conclusions: All three techniques estimated the BLDT to be below 2.5 seconds and achieved a remarkably close median value of 0.7 seconds. HSV appears to be the most precise method for measuring tear film surface quality. LSI appeared to be the most sensitive method for analyzing the tear film build-up.
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3373 - D959

Evaluating Tear Film Quality in Normal and Midly Symptomatic Dry Eyes With a Double-Pass Method

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Purpose: It is important the early detection of mild dry eye symptoms that could be aggravated by different treatments, for instance corneal refractive surgery. The purpose of this study is to evaluate and compare the tear film dynamics in normal healthy eyes and in eyes with mild symptoms of dry eye conditions. We applied a non-invasive method based on a double-pass instrument to characterize tear film quality.
Methods: The measuring technique consisted in the dynamic recording of double-pass retinal images (Santamaria, Artal & Bescos, JOSAA,1987) during unforced tear film break-up by using a clinical instrument (OQAS, Visiometrics SL, Spain). A symptomatic tear film produces a degraded retinal image and higher values of ocular scattering, quantified through an Objective Scatter Index (OSI). Two types of eyes were compared: a group (n=14) with mild dry eye symptoms and an asymptomatic control group (n=18). Series of 40 consecutive double-pass retinal images were recorded every 0.5 seconds, while the subject avoids blinking. Measurements were performed under low-light conditions to naturally increase pupil diameter maximizing the area analyzed for higher sensitivity. In every patient, additional clinical tests were also performed for comparison: break-up time (BUT), Schirmer I tests and a normalized questionnaire (McMonnies).
Results: From the temporal evolution of the OSI, it is possible to determine an objective parameter conceptually similar to BUT. This objective break-up time value (O_BUT) is set when OSI surpasses a defined threshold value as compared with the initial baseline. Symptomatic dry eyes showed larger variability of OSI over time as compared with the control eyes. In addition, the values of O_BUT were lower in the symptomatic patients and in some cases comparable to BUT estimates. Despite large individual variability, we found an average 90% OSI increase in dry eyes.
Conclusions: We applied a new objective optical method to evaluate the quality and stability of the tear film. It is sensitive to detect mild symptoms of dry eye and to differentiate from normal cases. The procedure allows early detection and follow-up of tear-film related patient's complaints.
CR: A. Benito, None; **M. Vilaseca**, Visiometrics SL, F; **S. Mirabet**, None; **G.M. Pérez**, None; **M.J. Romero**, None; **J. Pujol**, Visiometrics SL, F; Visiometrics SL, I; **J.M. Marín**, None; **J.L. Güell**, Visiometrics SL, I; **P. Artal**, Visiometrics SL, F; Visiometrics SL, P.
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3374 - D960

Serial Measurement of Tear Meniscus by Fourier-Domain Optical Coherence Tomography After Instillation of Artificial Tears in Patients With Dry Eyes

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Purpose: To use Fourier-Domain Optical Coherence Tomography to study the longitudinal effect of instillation of artificial tears on tear meniscus volume in patients with dry eyes.
Methods: Four patients with significant dry eyes were recruited in a consecutive manner from a tertiary cornea practice. The lower tear meniscus of the right eye in each subject was imaged by vertical scans centered on the inferior cornea and the lower eyelid using a Fourier-domain optical coherence tomography system (RTVue; Optovue, Inc., Fremont, CA) with a corneal adaptor. Two baseline measurements were taken for each subject prior to administration of a drop of artificial tears (Optive). Five serial pairs of measurements were then taken after the instillation of artificial tears at 1 minute, 2 minutes, 5 minutes, 10 minutes, and 15 minutes. Each measurement was taken two seconds after a blink. The lower meniscus height, depth, and angle were measured with a computer caliper. The cross-sectional area was calculated using a two-triangle approximation.
Results: The baseline tear measurements were 317 μm, 512.5 μm, 0.0695 mm2 for meniscus height, depth, and area respectively. At 1 minute after instillation of artificial tears the measurements increased by 212%, 488%, 2212%. The time to depletion of half of the gains in tear meniscus were 2.75 minutes, 4.75 minutes, and 2 minutes for height, depth, area respectively. The time to depletion of 75% of the tear meniscus gains was 3.5 minutes, 5.5 minutes, and 5.5 minutes.
Conclusion: Optical coherence tomography is able to quantify the increase in lower tear meniscus after installation of artificial tears in patients with dry eyes. This increase is transient, with a 50% reduction of both height and area within 3 minutes of drop instillation and a 75% reduction in all parameters by 6 minutes. Optical coherence tomography may serve as an invaluable tool in objectively quantifying the efficacy of dry eye treatments.
CR: M.C. Bujak, None; **D. Huang**, optovue inc, C; optovue inc, I; optovue inc, carl zeiss meditec inc, P; optovue inc, R; **S.R. Sadda**, heidelberg engineering, topcon medical systems, carl zeiss meditec, optovue inc, genentech, allergan, C; **Y. Li**, optovue, C; **P. Nguyen**, None; **R.K. Pappuru**, None; **S. Yiu**, None.
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3375 - D961

Comparation of Hygrometry With the Established Methods of Tear Film Measurement

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Purpose: To establish whether there is a correlation between dry eye symptoms, age and gender, a difference in symptom intensity in different times of the day, which test (Schirmer or TBUT) better correlates with dry eye sympoms, whether it is possible to reliably use cheap, easy to use hygrometer in everyday clinical setting, and finally whether there is statistically significant correlation between applied tests (TBUT, Schirmer and hygrometry).
Methods: Subjects: 45 in group without dry eye related symptoms, and 45 in group with symptoms. Examination included structured case history (questionnaire), slit lamp examination with fluoresceine staining, periocular hygrometry, TBUT test and Schirmer test.
Results: Statistically significant difference between groups was found regarding TBUT and Schirmer test values (P<0,001). No such difference was found regarding hygrometry values.
Conclusions: Hygrometry, as it was performed here, did not prove to be a reliable and accurate method in dry eye diagnostics. No statistically significant correlation was found between TBUT, Schirmer and hygrometry results in group with symptoms, so it may be concluded that these tests do not measure the same parameters of tear film function.
CR: I. Petricek, None; **A. Berta**, None; **J. Nemeth**, None; **M.T. Higazy**, None; **M. Prost**, None.
Support: None

3376 - D962

Correlation Between Optical Coherence Tomography Tear Meniscus Parameters and Schirmer's Test and Tear Break-Up Time

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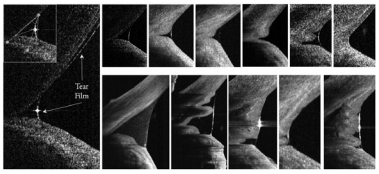
Purpose: To investigate the clinical utility of Fourier-domain optical coherence tomography (FD-OCT) measurements in the evaluation of keratoconjunctivitis sicca (KCS).
Methods: Patients with severe KCS requiring medical therapy (cyclosporine 0.05%) and/or occlusion were recruited. Instillation of all eye drops was stopped 2+ hours before measurements. An FD-OCT system (RTVue; Optovue, Inc., Fremont, CA) was used to image the lower tear menisci in both eyes. Vertical line scans were taken, centered on the inferior limbus. Software caliper was used to measure the meniscus height (H), depth (D) and angle at the inferior cornea-lid angle. Meniscus cross-sectional area (CS-A) was calculated using a two-triangle approximation. A certified technician conducted the 5-minute Schirmer's test with anesthesia and fluorescein tear break-up time (TBUT). Linear regression analysis was done to obtain the correlation (Pearson) between FD-OCT parameters and Schirmer's and TBUT.
Results: Twenty-one patients were recruited and 42 eyes imaged. Mean age was 57 (range 20-89), 80% were female, 65% Caucasian, 18% Asian, and 12% African-American. Figure 1 illustrates measurement techniques and the structural diversity of the angle. Fourteen eyes were excluded because of either conjunctival redundancy or poorly delineated meniscus. Mean TBUT was 4 minutes (range 1-7) and mean Schirmer's 9 mm (range 0-25). Mean CS-A was 0.035 +/-0.013 mm^2, H 0.288 +/-0.062 mm, D 0.160 +/-0.041 mm, and angle 30 +/-3 degrees; here, +/- denotes the 95% CI. Pearson coefficients are +0.40 for CS-A vs TBUT, -0.36 for angle vs TBUT, +0.12 for CS-A vs Schirmer's, and +0.26 for angle vs Schirmer's.



Conclusion: Correlation between Schirmer's and TBUT measurements and OCT was found. Tear meniscus formation and corneal wetability are both functions of tear properties. This may explain the higher correlation between CS-A and TBUT, as well as the negative correlation between angle and TBUT. These findings encourage further investigation.
CR: P. Nguyen, None; **D. Huang**, Optovue, Inc, I; Carl Zeiss Meditec Inc., I; Optovue, Inc, C; Optovue, Inc, P; Carl Zeiss Meditec Inc., P; Optovue, Inc, R; **S.R. Sadda**, Optovue, Inc., F; Carl Zeiss Meditec, F; Heidelberg Engineering, C; Genentech, C; Allergan, C; Topcon Medical Systems, P; Topcon Medical Systems, R; **R.R. Pappuru**, None; **S. Ramos**, None; **Y. Li**, Optovue, Inc., F; **S.C. Yiu**, None.
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3377 - D963

Evaluation of Enhanced OPI; Using Computerized Video Tracking of Tear Film Break Up for Evaluation of Tear Film Stability

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Purpose: Fluorescein imaging of tear film break up time (TFBUT) remains a standard diagnostic for assessing dry eye disease. However, this one-dimensional, singular assessment is a poor representation of overall tear film mechanics. Clinically, normal and dry eye subjects present overlapping ranges impeding clear differentiation between the populations. Instead, better discrimination between normal and dry eye subjects may be best observed in the events following TFBUT. The rapid rate of tear film decay in dry eye subjects exposes large areas of the cornea to environmental factors compared to normal subjects. To better assess the integrity of the tear film it becomes necessary to quantify both the time and area the cornea remains protect after TFBUT. This concept led to the development of a "time-area" protection assessment of the tear film as it breaks up within the subject's blink pattern under controlled visual task, functioning as an improved Ocular Protection Index (OPI).
Methods: Ten normal and ten dry eye subjects were video recorded for one minute OU in a one visit study. Inclusion was based on staining, symptoms, and a blink rate > 6 blinks/min. In order to carry out a real-time assessment of subjects' OPI, a computer program was developed. Fluorescein imaging videos are input into the program and graphical output of time-area of tear covered cornea is produced.
Results: Dry eye subjects had an average time-area protection of 95% ±4.7%; the normal population had coverage of 99.8% ±0.2% (p=0.0047). This means that, on average, dry subject expose about 5% of their corneal area to environmental factors, whereas subjects with stable tear film expose only about 0.2%.
Conclusion: This method of tear film assessment may be more clinically relevant and more precise then previous diagnostic techniques as it directly indicates the amount of corneal protection. The use of enhanced OPI may provide a more appropriate method to differentiate dry eye and non-dry eye subject populations, and evaluate potential therapeutic effects.
CR: E. Angjeli, Ora Inc., E; **J. Rodriguez**, Ora Inc., E; **K.J. Lane**, Ora Inc., E; **M.B. Abelson**, Ora Inc., E; **G.W. Ousler, III**, Ora Inc., E.
Support: None

3378 - D964

Magnetic Beads Produce Significant Artifacts in Luminex Tear Cytokine Assay

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Purpose: A Bio-Rad 27-Plex polystyrene bead cytokine assay was previously developed, validated and optimized for tears. A new magnetic bead version of the assay allows greater automation and removes filter plate factors that limit assay precision. This study applies the magnetic bead assay to tear cytokines.
Methods: Non-stimulated (NS) and stimulated tear samples were collected by micropipette from normal patients in sufficient quantity to run multiple aliquots on both the polystyrene and magnetic bead 27-Plex cytokine assay. An automated microplate washer was used, with filter plate and vacuum aspiration for the polystyrene bead assay, and plastic-based plate and magnetic plate holder for the magnetic bead assay. In a second study, 18 subjects collected 8 NS tear samples each over thirty days for polystyrene bead assay and 9 subjects collected 8 NS tear samples each over the same time-frame for magnetic bead assay. Samples were stored in assay buffer at -80°C prior to assay on a Luminex 200 system.
Results: Mean cytokine intra-assay coefficient of variation (CV) for tear sample replicates with the magnetic versus polystyrene bead assay was 8.1% vs. 17.4% for duplicates, 10.6% vs. 16.2% for triplicates and 9.8% vs. 16.0% for quadruplicates. In study 2, tear component interference was observed with the magnetic bead assay, in particular for eotaxin, IL-9, IL-17, IL-5 and TNF-□, with 25-fold higher levels relative to the polystyrene bead assay and much less sample to sample variation. Magnetic bead interference varied by subject, some showing even higher levels for many cytokines, others consistently eliciting almost undetectable levels of many cytokines.
Conclusions: While Luminex magnetic bead tear cytokine assays show improved intra-assay CVs, tear components appear to be interfering to produce falsely high cytokine values and reduced discrimination between tear samples for most subjects, while preventing detection of many cytokines in others. Luminex cytokine assay results for tears using magnetic beads should be treated with caution. Further validation studies are needed.
CR: R.J. Fullard, None; **L.E. Kehinde**, None; **K.S. Elder**, None; **M.K. Tran**, None; **N.M. Guyette**, None.
Support: UABSO Clinical Research Advisory Committee Grant

3379 - D965

Longitudinal Variability of Tear Film Osmolarity in Normal and Dry Eye Patients

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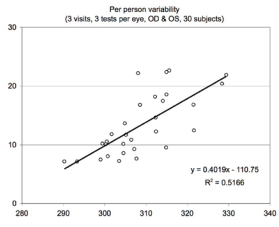
Purpose: Tear film hyperosmolarity and tear film instability are recognized as the two causative mechanisms of dry eye disease, yet the relationship between the signs are poorly understood. The purpose of this study was to evaluate the variability of OD vs OS tear film osmolarity relative to tear film instability in the diagnosis of dry eye disease.
Methods: Bilateral tear osmolarity was measured on three different days, with at least two weeks between each patient visit. 30 subjects were recruited for the study (n = 16 normal, n = 14 dry eye). Subjects included in the dry eye cohort were determined by an average osmolarity > 308 mOsms/L. At each visit, 50 nanoliters of tear fluid was simultaneously collected and analyzed (both OD and OS) by the TearLabTM Osmolarity System in triplicate.
Results: The average of normal subjects was 301.8±10.5 mOsms/L {range 290.2-307.7} while the average of hyperosmolar subjects was 315.6±18.6 mOsms/L {range 308.1-329.4}. The per-subject standard deviations were significantly different between the two groups ($p << 0.001$). Variability was strongly positively correlated with average osmolarity ($r2 = 0.52$), suggesting that tear film instability increased in dry eye disease. Of particular clinical interest, only 67% (56 of 84) of the tests performed on individual eyes were above 308 mOsms/L during the first measurement cycle on each day, whereas when the highest of either the OD or OS osmolarity result of an individual patient was considered, 81% of the dry eye subjects were correctly diagnosed during the first measurement cycle. By contrast 83% (80 of 96) tests on individual eyes were below 308 mOsms/L for normal subjects.



Conclusions: Variability in osmolarity is a hallmark of dry eye disease, reflecting the inability of the patient to maintain tear film homeostasis. It is recommended that physicians test both eyes to diagnose dry eye disease if the first value is less than 308 mOsms/L and the patient is symptomatic and/or dry eye disease is suspected.
CR: D.C. Eldridge, TearLab, Corp., C; **B.D. Sullivan**, TearLab, Corp., E; 7,017,394, P; **M.D. Berg**, TearLab, Corp., I; TearLab, Corp., E; **M.A. Lemp**, TearLab, Corp., I; TearLab, Corp., C; **D.S. Durrie**, TearLab, Corp., I.
Support: Alcon Laboratories

3380 - D966

Diagnostic Performance of Osmolarity Combined With Subset Markers of Dry Eye Disease in an Unstratified Patient Population

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Purpose: The purpose of this study was to evaluate whether the diagnostic performance of a novel, global test for dry eye disease (TearLab™ osmolarity) was improved by the addition of markers specific for aqueous deficient or evaporative dry eye.
Methods: Clinical signs were evaluated in both eyes of 299 subjects chosen from the general patient population (N = 82 Normal, N = 217 Dry Eye) across 11 sites in the EU and US. Diagnostic thresholds were defined as Osmolarity > 308 mOsms/L, TBUT <= 7 seconds, Schirmer < 7 mm, corneal staining > 0 and conjunctival staining > 0 in either eye. Sensitivity and specificity were calculated for each individual sign, as well as in combination with osmolarity through logical AND and logical OR functions.
Results: Individually, signs were ranked as follows: osmolarity (81%/80%; sensitivity/specificity), corneal staining (70%/82%), TBUT (79%/70%), conjunctival staining (86%/52%), and Schirmers (40%/82%). When required to exhibit both and elevated osmolarity AND one additional sign, the signs increased specificity at the expense of sensitivity: conjunctival staining (71%/93%), TBUT (62%/100%), corneal staining (59%/99%), and Schirmers (31%/99%). Similarly, when required to exhibit either an elevated osmolarity OR one other sign, the signs increased sensitivity at the expense of specificity: corneal staining (92%/63%), Schirmers (90%/63%), TBUT (98%/50%), and conjunctival staining (96%/40%). The best overall performance, as measured by the sum of sensitivity and specificity were osmolarity AND conjunctival staining (163%), osmolarity AND TBUT (162%), and then osmolarity alone (161%).
Conclusions: Although commonly used for diagnosis, markers that independently report the degree of lacrimal or meibomian dysfunction may misclassify hybrid forms of the disease. When combined with osmolarity, clinical signs of dry eye disease demonstrated improved overall diagnostic performance. However, the total sensitivity and specificity of combined testing was not substantially different than the diagnostic performance of osmolarity alone.
CR: B.D. Sullivan, TearLab, Corp., E; 7,017,394, P; **D.C. Eldridge**, TearLab, Corp., C; **M. Berg**, TearLab, Corp., I; TearLab, Corp., E; **V. Kosheleff**, TearLab, Corp., E; **A. Porreco**, TearLab, Corp., E; **J. Truitt**, TearLab, Corp., E; **M.A. Lemp**, TearLab, Corp., I; TearLab, Corp., C.
Support: Alcon Laboratories

3381 - D967
Tear Film Omolarity in Dry-Eye Disease

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Purpose: Dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface. Tear hyperosmolarity causes damage to the surface epithelium by activating a cascade of inflammatory events at the ocular surface and a release of inflammatory mediators into the tears (MAP kinases, NFkB signalling pathways) which leads to the generation of inflammatory cytokines (IL-1α, IL-1β, TNF-α) and Matrix Metalloproteinases (MMP 9). Epithelial damage involves cell death by apoptosis, a loss of goblet cells, the disturbance of mucin expression and an increased tear film instability which causes the exacerbation of ocular surface hyperosmolarity. Because of these pathogenetic mechanisms, tear film osmolarity could be an important diagnostic tool as indicator of ocular surface health in keratokonjunctivitis sicca and other ocular surface diseases. The aim of this prospective, non-randomized, clinical, single-centre study was to assess the changes in the osmolarity in tear samples of patients with keratokonjunctivitis sicca compared to healthy controls.
Methods: 28 patients (age 36±12, 10 males and 18 females) with severe keratokonjunctivitis sicca and 25 controls (age 42±18, 11 males and 14 females) were enrolled in the trial. Tear samples were collected from the inferior-temporal conjunctival sac. Inclusion criteria were a break up time (BUT) <5sec and a Jones-Test <5mm. Tear film osmolarity was analyzed by the OcuSense TearLab osmometer. A small nanoliter tear sample is sufficient (50 nl) for measurements. Statistical analyses were performed using Statistica™ software, p-values < 0.05 *, < 0.001** were considered significant.
Results: There was a significantly higher tear film osmolarity in patients with keratokonjunctivitis sicca (342±29.5 mOsmol/l) compared to the control group (302±18.2 mOsmol/l).
Conclusions: Tear film osmolarity can be determined using the OcuSense TearLab osmometer. Testing tear film osmolarity, applied alone or in combination, can be a very effective objective diagnostic tool in the diagnosis of dry eye disease (cutoff value for dry eye: 315.6 mOsmol/l).
CR: C. Jacobi, None; **F.E. Kruse**, None; **C. Cursiefen**, None.
Support: None

3382 - D968
A Unique Ocular Surface Interferometer (OSI) to Measure and Evaluate Lipid Layer Thickness (LLT)

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Purpose: To provide an ophthalmic imaging device intended for clinical use on dry eye patients to capture, archive, manipulate and store digital images of specular (interferometric) observations of the tear film, which can be visually and photographically monitored and numerically defined and documented.
Methods: The unique LipiView® Ocular Surface Interferometer (OSI) acquires the specularly reflected colors from the tear film. A spatially modulated light source allows removal of unwanted background images and stray light. Metrological RGB video data is acquired, frames and region of interest are selected automatically to remove blinks and artifacts, background images are removed, and statistical data are compared to a master look-up table (LUT) for lipid layer grading. The LUT is calculated using advanced principles of optical physics with commercial and custom modeling tools, using precise calibrations of the white light source and camera spectral responses, along with published refractive index and dispersion values for lipid and aqueous layers. This LUT has a spiral locus in RGB space. The processed output is expressed as Interference Color Units (ICUs), which correlate with Lipid Layer Thickness (LLT), using a kinemetric distribution. A previous report on the use of an early prototype OSI in routine clinical practice, demonstrated that LLT measurements correlate significantly with patient symptoms of dry eye.
Results: The processed instrument data matches well to the expected spiral of RGB values of the LUT, demonstrating agreement with theory. In addition a previous study on an early prototype OSI indicates the clinical suitability and applicability of this imaging technology.
Conclusion: The LipiView® OSI is viable as an objective tool for the evaluation of patient LLT. This imaging device is likely to significantly impact the routine clinical diagnosis and treatment monitoring of lipid deficient evaporative dry eye, since the methodology allows for ready incorporation of the LipiView® instrument to routine clinical practice.
CR: S.M. Grenon, TearScience, I; TearScience, E; TearScience, P; **D.R. Korb**, TearScience, I; TearScience, P; **C.A. Blackie**, TearScience, I; TearScience, E; **W. Weber**, TearScience, C; **R. Chinnock**, TearScience, C.
Support: None

3383 - D969
Changes in Human Meibum With Age and Meibomian Gland Dysfunction

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Purpose: Changes in the phase transition temperatures and conformation of human meibum lipid with age and meibomian gland dysfunction (MGD) have been quantified but with analysis of less than 1% of the infrared spectrum. The remaining 99 % of the spectra were further analyzed with principal component analysis (PCA) and confirm our previous studies as well as providing further insights into changes that occur in meibum with age, disease and therapy.
Methods: Infrared spectra of meibum from 41 patients diagnosed with MGD (Md) and 27 normal donors (Mn) were measured. Principal component analysis (PCA) was used to quantify the variance between the spectra.
Results: *Age related differences*. PCA was applied to a set of training spectra of human meibum to predict the age of meibum donors. The plot of predicted age versus actual age was linear, $p < 0.001$ with a slope of 1.00 and $r = 0.909$. This indicates that changes in factors of the meibum spectra (eigenvectors) were due to compositional differences with age. Eigenvector 1 accounted for 92 % of the variance between all of the meibum spectra. The spectral features of the two major eigenvectors indicate that with increasing age, the meibum contains more wax, double bonds and terminal CH_3 groups, and is less ordered. The environment of the carbonyl band becomes more hydrophobic with increasing age. These results are similar to those for human sebum. *Changes with MGD*. A training set of spectra were used to discriminate between Mn and Md with an accuracy of 93 %. This shows that eigenvectors contain compositional and structural information about the changes that occur with MGD. The spectral features of the major eigenvector indicate that Md contains more protein and cholesterol esters than Mn.
Conclusions: PCA is an excellent chemometric algorithm that may be used to diagnose and characterize MGD and age related changes in human meibum. With additional samples measured in the future, the training set will improve even more. PCA could be used by an untrained person as an initial diagnostic screening procedure for MGD. The eigenvectors that define the variations in the spectra with age and disease provide clues to the compositional and functional changes that occur in meibum with age and disease
CR: D. Borchman, None; **G.N. Foulks**, None; **M.C. Yappert**, None.
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3384 - D970
High Resolution Macroscopic (HRMac) Imaging of the Mouse Meibomian Gland

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Purpose: Recent studies suggest that the mouse meibomian gland (MG) develops atrophic changes with age similar to that of human. A better understanding of the structural changes that occur with age may provide important insights into age related MG dysfunction. The purpose of this study was to develop segmentation algorithms for the 3-dimensional reconstruction of the mouse MG that could then be used to volumetrically measure changes in gland structure.
Methods: Non-linear optical imaging combined with computer assisted array tomography was used to 3-dimensionally reconstruct the mouse MG. Mouse eyelids were fixed in 4% paraformaldehyde, embedded in LR White and serially sectioned. Sections were then scanned using a 20x objective and a series of tiled images (1.4 mm by 1.4 mm) with a resolution of 0.44 μm lateral and 3 μm axial were collected using a Zeiss 510 Meta LSCM and femtosecond laser to generate second harmonic (SHG) signals from collagen and two-photon excited fluorescence (TPEF) signals from cells. Image tiles were then digitally aligned, and the SHG signal used to outline and generate an MG mask used to make surface renderings and extract MG TPEF signals with Amira software.
Results: SHG was useful in clearly delineating the surface morphology (Fig. 1A) and extract the cellular TPEF signal (Fig. 1B) from MG.
Conclusions: Using HRMac imaging, 3-dimensional reconstructions of the mouse MG can be generated that can be used for measuring the volume of meibomian gland duct and acini.

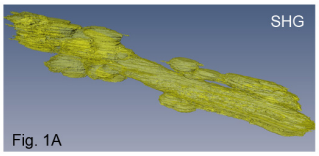

Fig. 1A

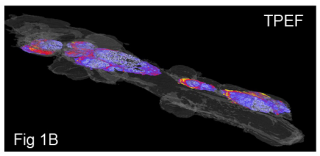

Fig 1B

CR: B.E. Jester, None; **C. Nien Shy**, None; **M. Winkler**, None; **J.V. Jester**, None; **D.J. Brown**, None.
Support: NEI grant EY016663, Research to Prevent Blindness Inc. and a gift from Alcon, Inc.

3385 - D971

Meibomian Gland Expression: Forces of Expression, Types of Secretion and the Limitation of Resulting Pain

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Purpose: Meibomian gland expression (MGE) is required for diagnosis of the presence or absence of individual meibomian gland (MG) secretion, the quality of the secretion, and the status of the MG excretory pathways. MG expression is the only method to determine the functionality of an individual MG. Instrumentation for standardized diagnostic expression utilizing 0.3PSI was introduced in 2008, and further custom instrumentation to exert pressures from 1.0 to 150 PSI was developed to determine the amount of force required to: (1) determine whether individual glands are functional when evaluated with forces approximating those of forceful blinking, (2) determine whether non-functional individual glands have the potential to secrete, (3) determine the forces required for therapeutic expression to treat obstructive MGD.
Methods: Expression pressures from in the range of 1 - 333 g/mm^2 (0.3 - 100 PSI) were applied for a standardized time of 15 seconds to evaluate the forces required for the aforementioned three forms of expression, and to study the nature of the expressed secreta resulting from a particular force.
Results: The force to evaluate gland functionality is 1 - 2 g/mm^2 (~ 0.3 - 0.6 PSI); to determine the potential to secrete 3 - 204 g/mm^2 (~ 0.9 - 60 PSI), and 4 - 275 g/mm^2 (~ 1.2 - 80 PSI) for therapeutic expression. The maximum force which could be tolerated, despite topical anesthesia, was limited by the subjective pain response, and varied from 15 - 275 g/mm^2 (~ 5 - 80 PSI).
Conclusions: Determination of MG functionality requires the evaluation of MG expressibility. The use of an instrument providing a standardized force for diagnostic expression is desirable, if not mandatory, for evaluation of MG function. Forces of significant magnitude are required for therapeutic expression, with pain being the limiting factor.
CR: C.A. Blackie, None; **D.R. Korb**, None.
Support: None

3386 - D972

In vivo Confocal Microscopy of Meibomian Glands in Sjogren's Syndrome

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Purpose: To evaluate the morphological changes of the meibomian glands and the status of periglandular inflammation in patients with primary (SSI) and secondary (SSII) Sjogren's syndrome by in vivo confocal microscopy and to investigate the correlations between clinical and confocal findings.
Methods: Twenty patients with SSI, 25 with SSII, 20 with meibomian gland disease (MGD) and 20 age- and gender-matched control subjects were consecutively enrolled. Each partecipant completed an Ocular Surface Disease Index questionnaire and underwent a full eye exam (including BUT, fluorescein and lissamine green staining, and Schirmer test) and a laser confocal microscopy examination of the meibomian glands (to study acinar unit density and diameter, meibum secretion reflectivity, atrophic and fibrotic changes and inflammatory cells density in basal epithelium, interstitium and glandular epithelium).
Results: All the clinical and confocal parameters showed statistical significant differences among the groups (P<0.001, Kruskal Wallis test). Confocal microscopy showed no differences between SSI and SSII (Mann-Whitney U test). Compared to control subjects, SS meibomian glands showed higher periglandular inflammation and secretion reflectivity and more evident atrophic and fibrotic changes (P<0.001, Mann-Whitney U test). Compared to MGD, SS meibomian glands had higher acinar density, smaller diameter, higher density of periglandular inflammatory cells and lower secretion reflectivity (P<0.001, Mann-Whitney U test). In SS patients, the 3 considered confocal signs of inflammation were significantly interrelated and correlated with corneal fluorescein staining (P≤0.01, Spearman). Acinar density and diameter were strongly correlated between them (P<0.001) and with BUT (P<0.05).
Conclusions: In vivo laser confocal microscopy can effectively demonstrate morphological and inflammatory changes of meibomian glands. In SS patients, the most evident alterations are inflammatory and fibrotic findings, with characteristics easily differentiable from MGD.
CR: E. Villani, None; **M. De Capitani**, None; **S. Beretta**, None; **D. Galimberti**, None; **F. Viola**, None; **V. Canton**, None; **R. Sala**, None; **R. Ratiglia**, None.
Support: None

3387 - D973

Changes in the Evaporation Rate of Tear Film After Digital Expression of Meibomian Glands in Patients With and Without Dry Eye

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Purpose: Dry eye (DE) has been divided into two different categories, evaporative and tear production-deficiency. During blinking, meibomian glands secrete lipids onto the preocular tear film. Changes in quantity or quality of the meibomian lipids affect the evaporation of the aqueous layer. This study was conducted to evaluate the effect of excess meibum in reducing the evaporation rate over time in patients with and without dry eye.
Methods: The effect of digital expression of meibomian glands on aqueous tear evaporation rate was tested in 11 normal subjects and 16 DE patients. DE group was divided into two groups based on clinical examination. Classic Keratoconjunctivitis Sicca (KCS) group with clear and easily expressed meibomian gland secretion and KCS with meibomian gland dysfunction (MGD) group with turbid and difficult to expressed meibomian gland secretion. Evaporative measurements were performed at baseline and following digital expression of both upper and lower lid meibomian glands. Each measurement took 10 minutes to complete and 2 minutes were allowed between measurements. A total of four sequential measurements were taken at 12, 24, 36 and 48 minutes following digital expression. An evaporometer (Oxdata, Portland, Oregon, USA) was used at two ranges of relative humidity (RH) 25% to 35% and 35% to 45%.The data was expressed in μl/cm^2/min.
Results: An increase in evaporation rate of the tear film was noted in all measurements using both RH in the classic KCS, and KCS with MGD groups compared to normals (p<0.05). The average evaporation rate at RH 25-35% and 35-45% was 0.056±0.016 and 0.040±0.008 for the classic KCS group; 0.055±0.026 and 0.037±0.019 for KCS with MGD; 0.033±0.012 and 0.023±0.008 for the normal group, respectively. In addition, a statistically significant decrease in evaporation rate was present in the normal and KCS with MGD groups between baseline and the first measurement following digital expression for both RH (p<0.05). There was no statistically significant difference between baseline and the other three measurements (p>0.05). The classic KCS group did not show a statistically significant change at any measurement following digital expression compared to baseline (p>0.05).
Conclusions: The Classic KCS and KCS with MGD patients showed an increase in tear evaporation rate compared to the normal group. The aqueous tear evaporation diminished in the normal and KCS with MGD groups following digital expression of the meibomian glands. Yet, this positive effect was transient and almost negligible after the second measurement.
CR: J.P. McCulley, Alcon, C; **J.C. Arciniega**, None; **J.C. Wojtowicz**, None; **E.M. Mohamed**, None.
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3388 - D974

The Influence of Eyelid Tension on Fluorescein Staining in Dry Eye

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Purpose: On the hypothesis that eyelid tension (LT) during blinking may influence the pathophysiology of ocular surface disorder; we investigated the relationship between LT and the localization of fluorescein staining (FS) in dry eye patients.
Methods: LT was measured in 130 eyes of 65 dry eye patients (D group) and 58 eyes of 31 normal controls (N group) using a specially designed blepahro-tensiometer, and the maximum and average eyelid tension (MLT and ALT) of the superior and inferior eyelids were determined as parameters of LT. FS of corneal and conjunctival epithelium was evaluated by regular clinical scores (0-3), by dividing the cornea into superior, middle and inferior regions, and the conjunctiva into superior, nasal/temporal interpalpebral, and inferior regions. SchirmerItest value, tear film breakup time were also examined.
Results: The LT values (mmHg) of the superior MLT, superior ALT, inferior MLT, and inferior ALT in the D group were 26.1± 5.9, 20.2 ± 5.7, 25.2 ± 7.1, 19.6 ± 6.6, and those in the N group were 21.5 ± 6.8, 16.2 ± 6.2, 21.0 ± 7.8 and 16.4 ± 6.8, respectively. The all of the four parameters of the D group were significantly higher than those of the N group ($p < 0.005$). The LT values of the N group significantly decreased with age, while those of the D group did not. FS scores for the inferior cornea and conjunctiva were well correlated with the inferior MLT and ALT, respectively ($p < 0.005$), and those for the superior conjunctiva were correlated with the superior MLT and ALT ($p < 0.05$) as well. No correlations were noted between any of the LT values and FS scores for the superior and middle cornea or the interpalpebral conjunctiva.
Conclusions: High level of LT may influence the degree of fluorescein staining by increased friction in dry eye patients.
CR: E. Noda, None; **M. Yamaguchi**, None; **A. Shiraishi**, None; **T. Kohno**, None; **M. Matsumoto**, None; **K. Ohta**, Menikon Corporation, P; **Y. Ohashi**, None.
Support: None

3389 - D975
Correlation Between Blink Rate, Tear Film Corneal Protection, and Corneal Staining

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Purpose: Increased blink rate may be an indication of ocular irritation and can function as a compensatory action in dry eye. Prolonged under-hydration of the corneal surface results in cellular shrinkage, observable by fluorescein staining, and can eventually lead to epithelial cell damage. However, the relationship between an increase in blink rate, the extent of corneal staining and the severity of tear film instability, assessed as corneal area protected by tear film within a undisturbed blink pattern, has not been clearly demonstrated.
Methods: Sixteen dry eye subjects were included in a one visit study based on staining symptoms, and a blink rate > 6 blinks/min. One minute video clips of blink patterns in the presence of staining were recorded then analyzed manually for presence of tear film break up. For grading purposes, the cornea was subdivided into 17 regions, with each region graded in a binary manner as either having an intact or disrupted tear film. An algorithm was developed for incorporating both a time and area component as an Ocular Protection Index (enhanced OPI). Total corneal staining was assessed using a 0-4 scale. The subject were divided into two groups for analysis; one with inter-blink interval (IBI)<5sec and IBI>5 sec.
Results: Subjects with a mean IBI<5 seconds had higher staining scores (sum=5.25, ±1.56) than subjects with a mean IBI>5 seconds (sum=3.96, ±1.49) (p=0.055). Individuals with a mean IBI<5 seconds also exhibited significantly greater corneal protection over the one minute video (area protected=93.4%, ±4.7%) than those with a mean IBI>5 seconds (area protected=88.5%, ±11.2%) (p=0.041).
Conclusion: This study demonstrates that those subjects with higher staining scores, are actively compensating with a shorter IBI and consequently have greater corneal coverage. Subjects with less staining have a less compensatory blink rate, and thus have lesser amounts of corneal protection. This suggests that low corneal protection precedes a severe dry stage, and lower IBI is a compensatory mechanism. In a drug development process, identification and control of these compensatory mechanisms becomes crucial for standardization.
CR: J. Rodriguez, Ora Inc., E; **E. Angjeli**, Ora Inc., E; **K.J. Lane**, Ora, Inc., E; **R. White**, Ora, Inc., E; **S. Breton**, Ora, Inc., E; **M.B. Abelson**, Ora, Inc., E.
Support: None

3390 - D976
Rose Bengal Staining Differentiates Primary and Secondary Sjogren's Syndrome and Keratoconjunctivitis Sicca

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Purpose: To compare the clinical presentation of 231 patients with primary Sjogren's syndrome (pSS), 58 patients with secondary Sjogren's syndrome (sSS) and 89 keratoconjunctivitis sicca (KCS) patients, to determine those procedures that best differentiate these groups in a multidisciplinary setting.
Methods: The records of all patients seen at the University Health Network Sjogren's Syndrome Clinic from October 1992 to July 2006 were reviewed and documented. Patients were diagnosed as primary and secondary SS by the American European consensus criteria (AECC) of 2002. Those patients seen at the clinic who were not diagnosed with SS and who had symptoms of dry eye and Schirmer scores of <= 10 mm in 5 minutes in at least one eye were included as aqueous deficient dry eye controls (KCS). There were 89 variables used in the analysis. Recursive partitioning was used to create a classification tree that demonstrated which characteristics best distinguished the three groups from each other.
Results: The presence of anti-ro antibodies was the most important variable in distinguishing the three groups. Rose bengal staining of 3/9 or greater and positive salivary flow results were the most important non-invasive variables that distinguished pSS, sSS and KCS.
Conclusions: Eye care practitioners can help to identify and differentiate pSS, sSS and KCS by using rose bengal staining and testing salivary flow.
CR: B. Caffery, None; **T. Simpson**, None; **S. Wang**, None.
Support: None

3391 - D977
Monitoring Corneal Density Changes Using Optical Coherence Tomography in the Animal Model for Keratoconjunctivitis Sicca

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Purpose: To use Optical Coherence Tomography (OCT) to observe changes in cell density underneath the corneal epithelium of eyes treated with trigeminal denervation. OCT imaging can be used to aid in the study of the effects of Neurotrophic keratopathy and Keratoconjunctivitis sicca.
Methods: Trigeminal nerve ablation surgery was performed at the V1-V2 junction using radiofrequency ablation to induce the symptoms associated with Neurotrophic Keratopathy and Keratoconjunctivitis Sicca. Denervation of the left trigeminal nerve isolated the intended effects of surgery to the left eye of the rat, while the right eye remained unaffected and served as the control. *Imaging using Optical Coherence Tomography was conducted on both eyes one day before surgery, and then 10 days and 17 days after surgery.* The OCT images were analyzed using Image J software for changes in gray value pixel intensity underneath the epithelium of the cornea to observe a change in the density of that layer. The changes in average pixel intensity from pre-treatment to 10 days and 17 days post-treatment were compared to observe the changes in cell density underneath the epithelium of the cornea with OCT imaging. Subtraction logic was used to control for confounding variables that may have affected the integrity of the rats' eyes between the imaging days by subtracting the light intensity of images of the control eye from the treated eye.
Results: Analysis of the OCT images shows a significant increase in pixel gray values of the images underneath the corneal epithelium 10 days after surgery, 0.890 (89.0%) and 17 days after surgery, 2.073 (207.3%) for the treated eye (p=0.036). Prior histopathologic data of the conjunctiva and the cornea after trigeminal denervation showed a decrease in goblet cells in the conjunctiva and the appearance of inflammatory cells beneath the corneal epithelium.
Conclusions: The results from the OCT images suggest significant changes in the cell density underneath the corneal epithelium after treatment. These changes imply degeneration of cells similar to the consequence of neurotrophic keratopathy and keratoconjunctivitis sicca. OCT imaging analysis serves as a noninvasive technique in further studying neurotrophic keratopathy and keratoconjunctivitis sicca.
CR: M. Ficinski, None; **K. Vanichsarn**, None; **Z. Deng**, None; **D. Tran**, None; **S. Tran**, None; **A. Ngo**, None; **T. Nguyen**, None; **L. Tran**, None; **Z. Chen**, None; **E.K. Wong, Jr.**, None.
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3392 - D978
Desiccating Stress Worsens Inflammation in an Alkali Burn Murine Model

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Purpose: The purpose of this study was to investigate the effects of concomitant dry eye and corneal alkali burn using a murine model.
Methods: C57BL/6 mice were divided in 2 groups: alkali injury (AI) or AI+ desiccating stress (AI+DS). Unilateral corneal alkali injury was created on a left eye of C57BL/6 mice, by placing a of 1N NaOH soaked 2.0mm diameter filter paper disc on the cornea for 10 seconds, followed by rinsing with balanced salt solution. Desiccating stress (DS) was induced by subcutaneous injection of scopolamine and exposure to a drafty low humidity environment for 5 days (DS5). The contralateral eyes served as controls: untreated (UT, no alkali injury, no dry eye), and DS5 (desiccating stress for 5 days). Mice were euthanized after 5 days and eyes and adnexae were collected for histology. Corneal frozen sections were immunostained for matrix-metalloproteinase 9 (MMP-9) and fluorescence intensity measured in the epithelium and in the stroma using NIS Elements Software.
Results: All eyes subjected to alkali burn and concomitant desiccating stress showed corneal perforation, and diffuse opacification of the surrounding cornea stroma 5 days post-injury. Eyes subjected to AI alone had different degrees of corneal opacification, but no corneal perforation. UT and DS5 eyes had no visible corneal opacification, nor perforation. Histologically, the corneas subjected to AI showed detached corneal epithelium, and moderate inflammatory cells in the corneal stroma. However, eyes subjected to AI+DS had central perforation and collapse of the anterior chamber, with iris and lens attached to the posterior cornea. This was accompanied by total loss of corneal epithelium and massive infiltration of corneal stroma by inflammatory cells that dissected tunnels in the cornea from the periphery. Immunostaining for MMP-9 showed that either DS alone or AI alone induced significant MMP-9 immunoreactivity in corneal epithelium (P=0.001 and P=0.006 vs. UT, respectively). The combination o f AI+DS induced a significant increase in MMP-9 reactivity in corneal stroma, to levels similar to the ones observed in the epithelium of DS or AI alone groups.
Conclusions: concomitant desiccating stress and alkali injury increased inflammatory cell infiltration and MMP-9 production in the cornea, leading to corneal perforation.
CR: C.S. De Paiva, None; **F.S.A. Pelegrino**, None; **A.J. McClellan**, None; **W.J. Farley**, None; **D.-Q. Li**, None; **S.C. Pflugfelder**, None.
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3393 - D979

Spontaneous Dry Eye Phenotype is Associated With Autoimmunity in Older Mice

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Purpose: Dry eye is a more prevalent disease in the elderly. The purpose of this study was to characterize the ocular surface and lacrimal gland changes that occur with natural aging.
Methods: Three age groups were evaluated: 8 week-old (8W), retired breeder (6-9 months-old, RB), and 24 month-old (24M) C57BL/6 mice. Eyes, adnexae, and extraorbital lacrimal glands were excised and prepared for either histology or frozen sections. Goblet cells (GC) were identified for counting with PAS staining. Corneal smoothness was assessed by reflection of a ring off the corneal surface. Immunohistochemistry was used to identify and count cells that were positive(+) for CD4, CD8, CD11b, CD11c, or IAIE in corneal and conjunctival tissue. The phenotype of antigen-presenting cells (APC) was assessed by dual immunofluorescent staining for CD11b or CD11c and IAIE in corneal sections.
Results: 24M mice spontaneously exhibit a dry eye phenotype compared to younger 8W mice, characterized by increased corneal irregularity, decreased conjunctival GC, and an increased number of CD4+, CD8+ , CD11b+, CD11c+, and IAIE+ cells in the conjunctival epithelium ($p<0.05$ for all). RB mice have a significant increase in corneal irregularity, decreased GC density,a decrease in the number of CD8+ cells and an increase in CD11b+ and IAIE+ cells in the conjuntival epithelium ($p<0.05$ for all). 24M mice also show a significant increase in IAIE+, CD11b+, and CD11c+ cells in the peripheral cornea ($p<0.05$ for all). While no change in CD11b+ cells was seen, a significant increase in IAIE and CD11c+ cells was noted for both RB and 24M groups in the central cornea ($p <0.05$ for both), compared to 8W mice. The majority of these CD11c+ cells also expressed IAIE.
Conclusions: Aging in mice was associated with increased corneal surface irregularity and decreased conjuntival GC density which is chartacteristic of dry eye. These changes were accompanied by immunopathological changes in the cornea and conjunctiva. These findings suggest that aging is associated with immune disregulation and autoimmunity that may promote the development of dry eye.
CR: A.J. McClellan, None; **W.J. Farley**, None; **S.C. Pflugfelder**, None; **C.S. De Paiva**, None.
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3394 - D980

Autoimmune Dacryoadenitis Induced in Rabbits by Intravenous Injection of Autologous Lymphocytes Activated Ex Vivo Against Lacrimal Antigens

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Purpose: Autologous peripheral blood lymphocytes (PBL), activated in a mixed cell reaction when co-cultured with purified rabbit lacrimal epithelial cells, induce a Sjögren's-like autoimmune dacryoadenitis when injected directly back into the donor animal's remaining inferior lacrimal gland (LG) or subcutaneously at a remote site. The purpose of the present study was to determine the ability of intravenously injected (IV) autologous stimulated lymphocytes to home to the LG and induce dacryoadenitis.
Methods: One inferior LG was surgically excised from each rabbit. Acinar epithelial cells were purified, cultured for 2 days, gamma-irradiated, and then co-cultured for 5 days with purified autologous PBL. Activated lymphocytes were used for autoadoptive transfer.
Results: Tear production was reduced 50% by 4 weeks and tear break up time was 70% less than normal. Ocular surface defects assessed by rose bengal staining were not as pronounced as after direct injection. However, 4 weeks after IV injection, unique areas of streaming lymphocytes were observed and lymphocytes were found close to interlobular and intralobar ducts. At 8 weeks LG showed clusters of abnormal lacrimal acinar cells and streaming lymphocytes. Immunohistochemical staining revealed that inflammatory infiltrates were composed of predominantly $CD4^+$ T cells.
Conclusions: Regardless of the injection site lymphocytes activated against lacrimal antigens can home to the lacrimal gland and are capable of inducing autoimmune dacryoadenitis, suggesting that the LG constitutively contains not only antigen presenting cells displaying potentially pathogenic autoantigen epitopes, but also chemokines and homing molecules that recruit $CD4^+$ T cells; we propose that these mediators normally recruit regulatory cells, but also recruit pathogenic effector cells that have been activated in peripheral lymphoid tissues.
CR: P.B. Thomas, None; **D.M. Samant**, None; **S. Selvam**, None; **D. Stevenson**, None; **J.E. Schechter**, None; **A.K. Mircheff**, None; **M.D. Trousdale**, None.
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3395 - D981

Immunopathogenesis in a Mouse Model of Lacrimal Keratoconjunctivitis is Antigen Specific

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Purpose: To determine if experimental ALKC is antigen-specific. We hypothesized that DO-11.10 DS pathogenic CD4+ T cells would not be able to adoptively transfer disease to nude T-cell deficient mice because the T cells from these mice would not be able to recognize an ALKC putative antigen (eg. Klk13).
Methods: Ova-TCR transgenic mice (DO-11.10) were exposed to desiccating stress for 10 days to determine if a dry eye response was elicited in these mice. BALB/c wild type (WT) or DO11.10 female mice were exposed to a desiccating environmental stress (DS; subcutaneous scopolamine injections, humidity <40%, and air flow across wire meshed screened cages) for 5 days. Control or 5 day DS mouse spleen and superficial cervical lymph node CD4+ T cells were IP injected into nude T cell deficient mouse recipients. Additionally, mice were treated with topical Klk13 or OVA. Tears and ocular surface tissues were collected for Luminex and histopathological analysis, respectively.
Results: Luminex analysis was done to determine tear cytokine levels from BALB/c WT or DO-11.10 donors and recipients. BALB/c WT mice exposed to 5 days DS had tear levels that were significantly higher than BALB/c WT control for IFN-γ, IL-12(p70), and TNF-α. These cytokines were not increased in the tears of DO-11.10 mice exposed to DS for 5 days. Adoptive transfer of 5 day DS BALB/c WT CD4+ T cells resulted in significantly higher levels of IFN-γ, IL-1α, and TNF-α. Adoptive transfer of DO-11.10 CD4+ T cells did not result in any statistically significant increases in cytokines in the tears. Inflammatory cell migration into the conjunctiva was only detected in recipient mice receiving BALB/c WT 5 day DS CD4+ T cells. Mice topically challenged with Klk13 displayed an ALKC phenotype. However, topical challenge with OVA did not induce ALKC disease pathology.
Conclusion: Ova-restricted T cells do not mediate ocular surface inflammation suggesting that experimental ALKC is antigen specific.
CR: K.F. Siemasko, Allergan, Inc., E; **C.S. Schaumburg**, Allergan, Inc., E; **M. Calonge**, Allergan, Inc., C; **V.L. Calder**, Allergan, Inc., C; **J.Y. Niederkorn**, Allergan, Inc., C; **C.S. De Paiva**, None; **S.C. Pflugfelder**, Allergan, Inc., C; **M.E. Stern**, Allergan, Inc., E.
Support: None

3396 - D982

The Functional Role of B Cells in a Mouse Model of Autoimmune Lacrimal Keratoconjunctivitis: Adoptive Transfer of Serum, but Not B Cells Alone, Mediates Ocular Surface Inflammation

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Purpose: To evaluate the functional role of B cells in the immunopathogenesis of experimental autoimmune lacrimal keratoconjunctivitis (ALKC).
Methods: Experiments were designed to determine if B cells are pathogenic in the context of ALKC by evaluating their i) role as antigen presenting cells (APC), ii) potential to mediate or augment ALKC and iii) putative role as autoantibody secreting cells. Experimental ALKC was induced by exposing female C57BL/6 wild-type (WT) mice to desiccating stress [DS: subcutaneous scopolamine (0.5 mg/0.2ml) TID, humidity <40%, and sustained airflow] for up to 3 weeks. To evaluate the role of B cells as APCs, control (CT) or 10 day DS-specific B cells and CT or DS CD4+ T cells purified from the cervical lymph nodes and spleen were co-cultured for 4 days; the CD4+ T cell proliferative response was measured by WST assay. Further, to determine if B cells or serum contribute to ALKC, DS-specific B cells (10 days DS) or DS serum (3 weeks DS) was adoptively transferred into nude recipient mice, which were subsequently evaluated for ocular surface inflammation 3 days post-transfer.
Results: CD4+ T cells purified from ALKC mice and co-cultured with DS-specific B cells (98% pure) displayed ~20% ($p=0.003$) more proliferation by WST than CD4+ T cell isolated from naïve mice. However, DS B cells did not mediate or augment ocular surface inflammation in nude recipient mice when adoptively transferred alone or when co-transferred with DS-specific CD4+ T cells. By contrast, nude recipient mice that received DS serum displayed a dose-dependent increase in tear cytokine levels of IL-1α ($p=0.03$), IL-1β ($p=0.03$) and TNF-α ($p=0.04$) compared to control mice.
Conclusions: These results indicate that DS-specific serum, but not B-cells alone, induces ocular surface inflammation in the context of the nude recipient mouse. Collectively, the data suggests that ALKC-specific autoantibodies may contribute to T cell-mediated immunopathogenesis of experimental ALKC.
CR: C.S. Schaumburg, Allergan, Inc., E; **K.F. Siemasko**, Allergan, Inc., E; **J. Gao**, Allergan, Inc., E; **L.A. Wheeler**, Allergan, Inc., E; **M. Calonge**, Allergan, Inc., C; **V.L. Calder**, Allergan, Inc., C; **J.Y. Niederkorn**, Allergan, Inc., C; **S.C. Pflugfelder**, Allergan, Inc., C; **M.E. Stern**, Allergan, Inc., E.
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3397 - D983
Expression of CCL3 and CCL4 in the Tear Film and Ocular Surface of Patients with Dry Eye Syndrome

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Purpose: To investigate the expression of CCL3/MIP (macrophage inflammatory protein)-1α and CCL4/MIP-1β in the tear film and ocular surface in dry eye syndrome.
Methods: Forty patients with dry eye (15 patients with Sjögren's syndrome and 25 patients with non-Sjögren's syndrome) and 10 control subjects were recruited. The concentrations of CCL3 and CCL4 in tears were measured using ELISA. The correlation between the levels and tear film and ocular surface parameters was analyzed. Expression of CCL3 and CCL4 was evaluated using immunohistochemistry. Flow cytometry was performed to analyze CCR5+ cells and their phenotypes in the conjunctiva.
Results: The concentrations of CCL3 and CCL4 were 141.51±71.91 and 426.37±298.72 pg/mL in dry eye patients and 26.43±36.67 and 4.30±8.64 pg/mL in the controls ($P<0.01$). The concentrations were significantly increased in Sjögren's syndrome patients compared with non-Sjögren's syndrome patients ($P<0.05$). The CCL3 and CCL4 levels correlated significantly with basal tear secretion, tear clearance rate, keratoepitheliopathy score, and goblet cell density ($P<0.05$). Staining for the chemokines increased in dry eye patients, especially in Sjögren's syndrome patients. Flow cytometry demonstrated an increased number of CCR5+ cells, CCR5+CD3+, and CCR5+CD14+ cells in dry eye patients.
Conclusions: Expression of CCL3 and CCL4, which attract monocyte, activated T cells, and NK cells, increases in the tear film and ocular surface of patients with dry eye syndrome, especially in those with Sjögren's syndrome. The level correlates with various tear film and ocular surface parameters.
CR: K.-C. Yoon, None; **J.-S. Lee**, None; **K.-H. Mun**, None; **I.-C. You**, None.
Support: None

3398 - D984
Time to Reflex Tearing in Response to Chemical Nasolacrimal Stimulus in Dry Eye Subjects versus Normals

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Purpose: We've previously shown the ability of the Controlled Adverse Environment (CAE model) to differentiate normals and dry eye patients based on time to reflex tearing. This research demonstrated a longer time to reflex tearing with increasing dry eye severity. Based on knowledge that nasal mucosal stimulation promotes tear production, the present study investigated the potential utility of chemical nasal mucosal stimulation in differentiating the two populations as well.
Methods: 11 normal subjects and 10 subjects diagnosed with dry eye were evaluated. After careful instruction to indicate the first sensation of tearing, an open vial containing the chemical stimulant, dilute ammonia, was introduced approximately 5 cm away from the subject's nose. A stopwatch was used to record the time from introduction of the chemical nasolacrimal stimulus to subject-reported tearing.
Results: Subjects with dry eye demonstrated a time to reflex tearing of 9.26 ± 4.54 seconds compared to normal values of 4.54 ± 2.20 seconds. The observed time to reflex tearing in subjects with dry eye demonstrated significantly longer time to reflex tearing compared to normals ($P = 0.017$).
Conclusions: These results demonstrated that subjects with dry eye exhibit a delayed reflex tearing response. These findings suggest that dry eye patients may have decreased protective mechanisms in response to chemical irritants. Furthermore, these findings may have implications for the impact of environmental irritants on subjects with compromised tear film barrier capabilities and warrant further investigation.
CR: C.J. Maffei, Ora, Inc., E; **G.W. Ousler, III**, Ora, Inc., E; **D.L. Welch**, Ora, Inc., E; **S.J. Curwen**, Ora, Inc., E; **E. Prifogle**, Ora, Inc., E; **M.B. Abelson**, Ora, Inc., E; Schepens Eye Research Institute and Harvard Medical School, E.
Support: None

3399 - D985
Lubricin Functions as an Ocular Surface Boundary Lubricant

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Purpose: Epithelial cells at the ocular surface are subject to significant shear forces (friction) generated during eyelid blinking as well as contact lens wear, especially in the presence of a compromised tear film. Given the recent discovery that ocular surface cells express mRNA of Lubricin, a cartilage boundary lubricant present in the knee, the purpose of this study was to assess the boundary lubricating ability of Lubricin at the cornea-eyelid biointerface.
Methods: Fresh human corneas and eyelids were mounted on a BOSE ELF3200 biomechanical testing machine with custom sample holders, forming a cornea-eyelid interface. Sample surfaces were articulated against each other at effective sliding velocities ranging from 0.3-30 mm/s under physiological loads of 15-20 kPa. Samples (n=6) were tested serially in lubricant baths of SterilePlus saline (Baush & Lomb), Aquify (CIBA Vision), and Lubricin purified from media conditioned by cartilage explants @ 300 ug/mL. Control samples (n=3) were tested in saline followed by bovine serum albumin (BSA) @ 300 ug/mL. Both static and kinetic friction coefficients were calculated.
Results: Lubricin functioned as an extremely effective friction-lowering boundary lubricant at the cornea-eyelid interface. At all sliding velocities, both static & kinetic frictions were greatest in saline, slightly decreased in Aquify, and lowest in Lubricin. Kinetic friction values were essentially invariant with sliding velocity, indicating a boundary mode of lubrication was operative, ranging from 0.28±0.02 (mean±sem) in saline to 0.23±0.02 in Aquify, to 0.15±0.02 in Lubricin. The friction lowering effect of Lubricin appeared specific, as BSA at 300 ug/mL did not reduce friction compared to saline.
Conclusions: These results indicate that Lubricin functions as an ocular surface boundary lubricant to reduce friction and wear, possibly better than currently available eye drops. These data support our hypothesis that Lubricin protects the ocular surface against significant shear forces generated during an eyelid blink and contact lens wear.
CR: T.A. Schmidt, Singularis, Inc., I; Singularis, Inc., P; **D.A. Sullivan**, Singularis, Inc., I; Singularis, Inc., P; **E.R. Truitt, III**, Singularis, Inc., I; Singularis, Inc., P; **B.D. Sullivan**, Singularis, Inc., I; Singularis, Inc., P.
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3400 - D986
Evaluation of Biofilms on Punctal Plugs and Intracanalicular Devices

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Purpose: Punctal occlusion is commonly used in the management of dry eye syndrome. Recent studies have demonstrated an association of punctal plugs and intracanalicular devices with biofilm formation and clinical infection. The purpose of our study is to evaluate these devices for biofilm formation using a novel culture technique and to further characterize the microorganisms that form these biofilms.
Methods: Patients at the University of North Carolina Ophthalmology clinic underwent removal of punctal plugs and intracanalicular stents for routine clinical indications over a nine-month period from December 2008 to September 2009. The devices were submitted for microbiology culture before and after water-bath sonication to loosen adherent microorganisms. Main outcome measures were clinical signs and symptoms of lacrimal infection, culture positivity, identification of isolated microorganisms, and enhanced recovery of microorganisms from culture after sonication. This is a prospective, observational study.
Results: 54 patients were included in the study (40 women, 14 men; average age 58 years ± 16.1 [SD] years). 86 devices (81 punctal plugs and 5 monocanalicular stents) were submitted for culture. The devices were in place an average of 76.5 days ± 53.3 [SD] days. None of the patients had clinical evidence of lacrimal infection. Cultures were positive in 36 of 86 samples (42%). 56% were positive for oropharyngeal flora. 19% were positive for skin flora. Gram negative rods were isolated in 14% of samples. Anaerobes and *Stenotrophomonas maltophilia* were each isolated in 11% of samples. Nocardia was isolated in 6% of samples. One sample grew *Mycobacterium chelonae*. In 5 of the 86 samples (6%), sonication enhanced the recovery of microorganisms from culture by a ten-fold or more increase in the number of colony-forming units.
Conclusions: Punctal plugs and intracanalicular devices can become colonized with bacteria and form biofilms composed of oropharyngeal flora, skin flora, and other rare but clinically significant pathogens. Water-bath sonication did not aid in the enhanced detection of biofilms.
CR: N. Esmaili, None; **K. McCall-Culbreath**, None; **P. Gilligan**, None; **C. Fowler**, None; **A. Fowler**, None.
Support: None

3401 - D987
Changes in Gene Expression in Meibomian Gland Dysfunction

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Purpose: Meibomian gland dysfunction (MGD) is believed to be the leading cause of dry eye syndrome throughout the world. However, the precise mechanism(s) underlying the pathogenesis of this disease are unclear. In this study we sought to identify meibomian gland genes that may promote the development and/or progression of human MGD.
Methods: Lid tissues were obtained from male and female MGD patients and age-matched controls (n = 6/group) after eyelid surgeries. Meibomian glands were isolated and then processed for RNA extraction and the analysis of gene expression by using Illumina humanHT-12 v3 expression beadchips. These chips target more than 25,000 annotated genes and contain over 48,000 probes. Standardized data were generated with Illumina BeadStudio software, background subtraction and cubic spline normalization, and analyzed with GeneSifter.Net bioinformatics and statistical software.
Results: Our results show that MGD is associated with significant ($p < 0.05$) alterations in the expression of almost 400 genes in the human meibomian gland. The levels of 197 transcripts, including small proline-rich proteins 3, 2A and 2F, cystatin A, keratins 10, 6B and 6C, and S100 calcium binding proteins A7, A8 and A9, were significantly increased, while the expression of 194 genes, such as for alpha polypeptide platelet-derived growth factor receptor, was significantly decreased. These changes were accompanied by alterations in many gene ontologies, including an elevation of biological processes for keratinocyte differentiation and ectoderm development, and a suppression of others like cell growth. In addition, MGD was associated with a significant rise in pathways linked to deoxyribonuclease and structural molecule activities and cornified envelope components, and a reduction in those related to cytoplasmic vesicles.
Conclusions: Our findings demonstrate that MGD is accompanied by multiple changes in gene expression in the meibomian gland. The nature of these alterations, including the upregulation of genes encoding small proline-rich proteins and S100 calcium binding proteins, suggest that keratinization and inflammation may play important roles in the pathogenesis of MGD.
CR: S. Liu, None; **S.M. Richards**, None; **D.A. Sullivan**, None.
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3402 - D988
A Genetic Association of IL 6 and IL6R Genes in Korean Dry Eye Patients

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Purpose: To determine the possibility of inflammation related genes, interleukin 6 (IL6) and interleukin 6 receptor (IL6) genes, as potential susceptibility candidate gene for Korean patients with dry eye, we investigated the association of the IL6 and IL6R polymorphisms in unrelated Korean patients with dry eye.
Methods: Genomic DNA was extracted from blood samples of unrelated Dry eye patients with two symptoms of non-Sjogren's disease and Sjogren's disease, visited the Department of Ophthalmology at the Catholic University Medical Center. To screen genetic variations in rs1800795 of IL6 promoter region and Asp358Ala (rs8192284) of IL6R were performed using polymerase chain reaction, restriction fragment length polymorphism and direct sequencing. Control individuals were selected from the general population without dry eye.
Results: In this study, we investigated rs1800795 of IL6 and rs8192284 of IL6R in Korean patients with dry eye. Genotypic and allelic distribution of the rs8192284 of IL6R was significantly different between dry eye patients with non-Sjogren's symptom and controls; C allele of dry eye patients was significantly difference compared with control subjects ($p = 0.045$, O.R. = 1.902). And also, Sjogren's disease patients had significantly higher C allele frequency, C allele had significantly higher than controls was significantly difference compared with control subjects ($p<0.001$, O.R.=2.959). But there were no statistically significant differences in the allele and genotype frequencies of rs1800795 of IL6 promoter region between dry eye patients and controls. The genotype distributions of all polymorphisms of IL6 and IL6R among the control subjects and the affected individuals were in Hardy-Weinberg equilibrium.
Conclusions: This is the first report of *IL6 and IL6R* gene variations screening in Korean dry eye patients. Significant differences in allelic frequency in rs8192284 of the *IL6R* gene between dry eye, which is non-Sjogren's and Sjogren's diseases, and the control group suggest that *IL6R* polymorphisms may play a role in the susceptibility of unrelated Korean to develop dry eye, particularly, Sjogren's disease.
CR: S. Choi, None; **K. Na**, None; **J. Mok**, None; **J. Park**, None; **S. Chung**, None; **C.-K. Joo**, None.
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3403 - D989
Increased Responsiveness of Corneal Cold Receptors in an Experimental Model of Dry Eye

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Purpose: To evaluate the effect of decreased tear secretion on nerve impulse activity of corneal cold thermoreceptors in an animal model of dry eye.
Methods: Main lacrimal gland was surgically removed in 9 guinea pigs. 18 animals served as a control group. One week afterwards, the cornea was excised, placed in a recording chamber and superfused continuously with physiological solution. Single nerve terminal impulses of cold thermoreceptors were recorded using a glass pipette applied onto the cornea and conventional recording equipment. Spontaneous activity (SA) at 34-36°C was first recorded, followed by cooling pulses to 22-25°C, changing the temperature of the perfusing solution at variable rates. Mean frequency of spontaneous activity (SA), peak frequency value (PF) during the cooling pulses and temperature at the peak firing response (PT) were analyzed. Multiple regression analysis was applied to evaluate the effect of basal temperature, basal frequency and cooling velocity on cooling responses.
Results: Tear secretion decreased compared to baseline after surgery (2.3±1.1 mm vs. 17.6±4.3 mm, $p<0.05$). 97 cold nerve terminals were analysed in the dry eye and 74 in the control group. SA at 32-36°C was increased in dry eye corneas (10.2±0.6 Hz) compared to controls (7.9±0.4 Hz, $p=0.003$). Significant correlation was found between basal frequency and PF in both groups (dry eye: $r=0.61$, control: $r=0.72$, $p<0.05$), and between cooling velocity and PF in both groups (dry eye: $r=0.69$, control: $r=0.51$, $p<0.05$). Multiple regression analysis showed significant effect of surgery on PF ($p=0.006$) and PT ($p<0.001$). PF in response to cooling was significantly higher (16.1±0.9 Hz) in corneas of dry eyes than in control corneas (11.9±0.7 Hz, $p=0.006$). The temperature decrease required to reach the maximum response was 6.2±0.3 °C in the dry eye group and 6.8±0.5 °C in the control group ($p<0.001$).
Conclusions: Decreased tear secretion induced by removal of the main lacrimal gland altered the response characteristics and sensitivity of corneal cold nerve terminals. Increased nerve impulse activity in this population of sensory afferents may contribute to dryness sensations in dry eye pathologies.
CR: I. Kovacs, None; **S. Quirce**, None; **C. Luna**, None; **M.C. Acosta**, None; **C. Belmonte**, None; **J. Gallar**, None.
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3404 - D990
Co Morbid Pain in the Innervation Territories of the Non-Ophthalmic Branches of the Trigeminal Nerve Triggered by Corneal Neuropathic Pain Can Be Mitigated by Modulators of Corneal Nociceptor Na^+ Channel Function

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Purpose: To report the existence of cornea-centric trigeminal neuropathic pain syndrome.
Methods: In this retrospective case series, 26 patients with corneal neuropathic pain referred for scleral lens fitting during the period 11/01/08-10/30/09 who failed to experience sufficient benefit wearing the Boston Ocular Surface Prosthesis (BOS-P) were identified. Of these, 10 patients reported collateral head, orbital, facial and temporomandibular pain. After informed consent for off-label drug use, their corneas were treated with subanesthetic concentrations of an amide Na^+ channel blocker (lidocaine or ropivacaine) or lacosamide, a non-anesthetic Na^+ channel modulator. Either drug was delivered in the fluid reservoir of the BOS-P. Patients were closely monitored by slit lamp biomicroscopy, HRT-II laser confocal microscopy, and Cochet-Bonnet esthesiometry. Pain metrics prior to and during treatment were recorded.
Results: Patient mean age was 49.2±11.1 yrs (2 male, 7 female). Mean average eye pain score prior to treatment was moderate to severe and ranged from mild to excruciating. No metric was applied to co morbid pain prior to treatment. The commonest co morbid pain symptom was migrainous type headaches. Treatment of the cornea with either subanesthetic concentrations of a Na^+ channel blocker or a non-anesthetic Na^+ channel modulator relieved symptoms of corneal pain/photophobia and collateral pain involving the head, orbit, face and/or jaw in 6 (60%) patients. 1 (10%) of these 6 patients reported only temporary relief and 4 (40%) patients did not experience any relief during treatment. Average treatment time for patients experiencing relief was 5.2±2.7 months.
Conclusions: Downregulating the activity of the pathologically sensitized corneal nociceptors by sodium channel blockade or modulation can be effective in mitigating symptoms of corneal and collateral neuropathic pain in some patients. Failure of this topical treatment may indicate dysfunctional nociceptive activity in the more central corneal pain signaling pathway and may require concomitant use of systemic analgesic therapy.
CR: P. Rosenthal, None; **J. Bradley**, None.
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3405 - D991
Effects of the TRPM8 Agonist Menthol on Corneal Sensitivity and Tear Secretion

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Purpose: To define the spontaneous and stimulus-evoked changes in corneal sensitivity following topical application of menthol.
Methods: Corneal esthesiometry was performed in 10 volunteers of both sexes (6 male, 4 female; 22±1 years), in 4 separate sessions using a Belmonte gas esthesiometer (Deriva Global SL, Spain), before and 30 min after application to one eye of a menthol solution (60μl; 1, 10 or 100μM menthol, and vehicle). The attributes of the sensation evoked by menthol (cooling, warmth, discomfort, irritation, burning, stinging, pricking, itching, tingling, numbing pain) were rated independently in 0-10 visual analogue scales (VAS). The Schirmer test was applied before and after menthol. For corneal sensation measurements, mechanical (air at 0-200ml/min flow), chemical (0%-80%CO_2 in air at subthreshold flow) and cold (air at subthreshold flow reducing corneal temperature from -0.1° to -4.5°C) stimuli were applied to the center of the cornea. After each stimulus, the magnitude of the intensity of the evoked sensation and of several of its psychophysical attributes (irritation, pricking, burning, warm, cold) was scored in separate VAS scales. Experiments were double-blinded.
Results: After menthol instillation highest scores were given to irritation, cold, discomfort and stinging sensations. Intensity of the sensation evoked by corneal mechanical and chemical stimulation was not significantly modified by menthol, but perceived irritation was decreased, particularly after the highest menthol concentration. Intensity of the sensation evoked by cold stimulation was increased by menthol in a dose-dependent manner. Tear secretion was not modified by menthol application.
Conclusions: Mild irritation and discomfort were the main spontaneous sensation parameters evoked by topical application of menthol to the eye. In addition, menthol attenuated the irritation sensations evoked by mechanical and chemical stimulation of the cornea and enhanced intensity of the sensation evoked by cold. This possibly reflects an increase by menthol of spontaneous and stimulus-evoked activity in cold receptor fibers and a partial desensitization of nociceptors, and suggest that augmented cold receptor activity evokes conscious sensations with an unpleasant component.
CR: J. Belmonte, None; **S. Alfaro**, None; **T.B. Berenguer**, None; **J.M. Costa**, None; **V. Cuquerella**, None; **M.C. Acosta**, None; **C. Belmonte**, None; **J. Gallar**, None.
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3406 - D992
FK962 Enhances Axonal Regeneration in Cultured Rat Trigeminal Ganglion Cells

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Purpose: Innervation of ocular tissues by central and peripheral nerves is essential for normal physiological function. Disruption of this innervation causes serious ocular diseases. For example, amputation of trigeminal nerve leads to decreased corneal sensitivity and dry eye, and degeneration of the optic nerve from high intra-ocular pressure leads to profound visual defects. N-(1-acetylpiperidin-4-yl)-4-fluorobenzamide (FK962) causes release of neurotrophic factors, and is a putative cognitive enhancer. The purposes of the present experiments were to determine if: 1) FK962 induces axonal elongation in cultured trigeminal ganglion cells, and 2) FK962 lengthens axons in an *in vitro* model of axotomy.
Methods: Trigeminal ganglion cells (neuronal + Schwann cells) or isolated neuronal cells were cultured for 24 hours with or without FK962. Cells were fixed, labeled with antibody for neurofilament and substance P, and observed with a fluorescence microscope. An axotomy model was produced by culturing trigeminal ganglion cells with nerve growth factor (NGF) for 48 hours. Axons were then transected with tweezers, cultured with FK962 for an additional 24 hours, and the cells were immunostained as above.
Results: In cultured neurons, FK962 induced sprouting and elongation of axons co-cultured with or without Schwann cells. In the axotomy model without added FK962, axons distal to the transected nerve terminals rapidly disappeared, and with cultured time, the axons then regenerated. Addition of FK962 further enhanced regeneration of axons in the axotomized trigeminal ganglion cells.
Conclusions: FK962 enhanced sprouting and regeneration of axons in both normal and transected axons. The effect of FK962 is at least partially due to direct action on neurons. Concomitant indirect action by FK962 on Schwann cells may also be important for supporting the neurons.
CR: Y. Kishimoto, Senju Pharmaceutical Co., Ltd., E; **C. Yabuta**, Senju Pharmaceutical Co., Ltd., E; **M. Azuma**, Senju Pharmaceutical Co., Ltd., E.
Support: None

3407 - D993
Topical Application of FK962 Enhances Axonal Elongation and Corneal Sensitivity in a Rabbit Model of LASIK Surgery

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Purpose: Laser in situ keratomileusis (LASIK) refractive surgery has been well accepted for treatment of myopia. However, creation of the corneal flap causes nerve degeneration and decreases corneal sensitivity, leading to dry eye and neurotrophic epitheliopathy. Our previous study showed that somatostatin accelerated recovery of corneal sensitivity in a rabbit model of LASIK surgery. FK962 is an enhancer of somatostatin release, and it improved memory deficits in animal models. The purpose of the present experiment was to test if FK962 facilitates axonal elongation and recovery of corneal sensitivity after creation of a corneal flap in rabbits.
Methods: One 130 μm-thick by 8.5 mm-diameter flap was created on each rabbit cornea, and 1 μM FK962 was applied topically 4 times per day. Clinical studies previously found that decreased corneal sensitivity and concomitant complications were usually observed one week after surgery. Corneal sensitivity in rabbits was thus measured 8 days after flap creation using Cochet-Bonnet esthesiometer. Whole-mount corneal sections were prepared and immunolabeled with anti-neurofilament antibody to visualize transected nerves. The elongated axons from the transected nerve terminal were scored to assess re-innervation. Rabbit trigeminal ganglion cells were also cultured for 48 hours with FK962 to confirm axonal elongation *in vitro*.
Results: In control animals, distal axons from transected nerve terminals disappeared soon after flap surgery, and with time, axons then regenerated and elongated. Increasing corneal sensitivity was well associated with axonal elongation suggesting functional re-innervation. Topical application of FK962 for 7 days significantly enhanced elongation of axons toward corneal sub-epithelial region compared to controls. Corneal sensitivity was also significantly enhanced by FK962. FK962 also significantly increased sprouting and elongation of axons in cultured trigeminal ganglion cells.
Conclusions: The results from our rabbit model showed that topical application of FK962 facilitated corneal re-innervation and recovery of sensitivity. We speculate that topical application of FK962 may decrease complications in patients after LASIK surgery.
CR: T. Oka, Senju Pharmaceuticals, E; **F. Yano**, Senju Pharmaceuticals, E; **Y. Tamada**, Senju Pharmaceuticals, E; **C. Yabuta**, Senju Pharmaceuticals, E; **M. Azuma**, Senju Pharmaceuticals, E.
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