**Program Number: 4722**
**Presentation Time:** 3:45 PM–4:00 PM

Resolvins D2 increases conjunctival goblet cell intracellular [Ca²⁺] by increasing cellular cAMP to activate protein kinase A

**Purpose:** Specialized substances of resolution (SPMs) such as the resolvins, lipoxins, and protectins function to actively resolve pathological inflammatory processes. Another critical function is to maintain tissue homeostasis in health. The purpose of this study was to determine if an SPM of the resolvin (Rv) family, RvD2, regulates conjunctival goblet cell (GC) function and uses the same cellular signaling pathways as observed with RvD1, RvE1, and lipoxin A₄.

**Methods:** Rat conjunctival GCs were grown from tissue explants and first passage cells were used. Presence of the RvD2 receptor GPR18 was determined by RT-PCR. To measure intracellular [Ca²⁺] ([Ca²⁺]ᵢ) cells were incubated with the calcium indicator dye fura2 and [Ca²⁺]ᵢ was determined by RT-PCR. To measure intracellular [Ca²⁺]ᵢ first passage cells were used. Message for the RvD2 receptor GPR18 was expressed to the same magnitude as RvD1 with [Ca²⁺]ᵢ to the same magnitude as RvD1 with 10⁻⁸ M giving the maximal response for both Rvs. The protein kinase A inhibitor H-89 blocked the RvD2, but not the RvD1, stimulated increase in [Ca²⁺]ᵢ. Blockage of the response to VIP was the positive control. Forskolin that increases cellular cAMP levels independent of receptor stimulation and IBMX that prevents breakdown of cAMP each significantly increased [Ca²⁺]ᵢ. Removal of extracellular Ca²⁺ and inhibition of the IP3 receptor with 2-APB blocked the RvD2 stimulated increase in [Ca²⁺]ᵢ. This finding suggests that RvD2 uses IP3-dependent intracellular Ca²⁺ stores similarly to the positive control carbachol.

**Results:** Unlike RvD1, RvE1, and lipoxin A₄, RvD2 uses the cAMP/protein kinase A signaling pathway to increase intracellular Ca²⁺ levels and could play a role in maintaining conjunctival GC homeostasis in health.

**Commercial Relationships:** Darlene A. Dartt, None; Nora Botten, None; Robin R. Hodges, None; Dayu Li, None; Jeffrey Bair, None; Marie Shatos, None; Tor Utheim, None; Charles Serhan, None; Wei Li, None

**Support:** NIH Grant EY019470 and NIH Grant GM38765

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**Program Number: 4723**
**Presentation Time:** 4:00 PM–4:15 PM

**Pathological changes of meibomian gland and ocular surface in Apolipoprotein E knockout mice**

**Purpose:** To investigate the pathological changes of meibomian gland (MG) and ocular surface tissues in Apolipoprotein E knockout (ApoE⁻/⁻) mice, and to illustrate the effect of ApoE on meibomian gland function.

**Methods:** The ocular surface of ApoE⁻/⁻ male mice (n=30) aged from 3 months to 7 months, and age and sex matched wild type mice (n=30) was observed under slit lamp microscope. MG tissue sections underwent H&E staining, Oil Red O staining, TUNEL assay, and immuno-fluorescence staining for K10, Fabp5, Ki67, K6a, p63, PPAR-gamma, NF-kB p65, p-NF-kB p65, caspase 3, 8, and immune-histochemical staining for CD45. Real-time RT-PCR and Western blot was performed to detect above mentioned gene expression in MG tissues. Lipid metabolism related genes expression in MG were also detected by real-time RT-PCR.

**Results:** Eyelid thickening, keratinization and corneal neovascularization occurred in ApoE⁻/⁻ mice at the age of 5 months, and the changes were more obvious at 7 months. H&E staining showed hypertrophy of the meibomian gland acinus, and Oil red O staining showed accumulation of lipid in MG acinus of ApoE⁻/⁻ mice. Both K10 and Fabp5 expression were increased, while Ki67, K6a, p63 were decreased in ApoE⁻/⁻ mice compared to the wild type mice. Cytoplasmic and nuclear expression of PPAR-gamma was decreased, NF-kB p65, p-NF-kB p65, caspase 3, and caspase 8 expression were higher in the ApoE⁻/⁻ mice compared to the wild type mice. TUNEL assay showed more positive cells in the meibomian gland acinus of ApoE⁻/⁻ mice. CD45 positive cell infiltration increased from 5 months to 7 months in ApoE⁻/⁻ mice. Lipid metabolism related genes such as FFA1, PAR3, ACSL3, and LIPC were decreased in ApoE⁻/⁻ mice compared to the wild type mice.

**Conclusions:** ApoE knockout mice represented abnormal meibomian gland acinar cell proliferation, differentiation, and lipid metabolism, they also showed eyelid and corneal pathological changes. These mice may be used as model to study the pathophysiology of meibomian gland dysfunction.

**Commercial Relationships:** Jinghua Bu, None; Yang Wu, None; Xin He, None; chengyou zuo, None; Shangkun Ou, None; ZUGOU LIU, None; Wei Li, None

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We have shown that progenitor cell engraftment is more efficient during LG regeneration phase vs acute inflammation phase. The induction of inflammation in the LG is facilitated by extracellular ATP and mediated by the pannexin-1 (Panx1) membrane channel glycoprotein. We hypothesized that suppression of inflammation through manipulation of Panx1 pathway activity can stimulate epithelial cell progenitors (EPCPs) engraftment.

**Methods:** The expression of pannexins in mouse LG was assayed by qRT-PCR, immunohistochemistry and RNA-seqencing. Acute LG inflammation was induced by intraglandular injection of 1 μg interleukin-1 alpha (IL1α). Thrombospondin-1-null (TSP-1-null) mouse was used as a model of chronic LG inflammation. EPCPs transplantation was performed to study cell engraftment. Panx1-specific blocking peptide (panx (100 μm, sequence WRQAFAFDYSY) prior to EPCP cell transplantation. Cell engraftment and area of inflammation were analyzed by microscopy. The unpaired two-tailed Student’s t-test was used to determine statistical significance.

**Results:** Two pannexin isoforms, Panx1 and Panx2 were detected in the LG epithelial cells at both mRNAs and protein level. In the acute model of regeneration, Panx1 and pro-inflammatory cytokines and caspases 1 and 4 were strongly upregulated during the inflammatory phase, 1-3 days after IL-1 injection. In contrast, Panx2 was increased only during LG regeneration. The analysis of EPCP engraftment showed a significant and reproducible positive correlation between the (panx peptide treatment and the number of engrafted cells per cross section, with an average increase of engrafted cells of approximately 63% relative to untreated controls. The same strategy showed efficacy in the TSP-1-null mouse chronic model: blocking inflammation by LG treatment with either (panx peptide or by silencing caspase 4 using dsRNAi showed a significant decrease of total area (cumulative foci) affected by inflammation.

**Conclusions:** Our results suggest that the manipulation of Panx1 and/or Caspase4(11) is a new beneficial strategy to enhance donor cell engraftment and LG regeneration through the reduction of inflammation.

**Commercial Relationships:** Helen P. Makarenkova, None; Anastasia Gromova, None; Xin Tang, None; Driss Zoukhri, None; Valery I. Shestopalov, None

**Support:** NIH NEI Grant EY021292

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**Program Number:** 4726
**Presentation Time:** 4:45 PM–5:00 PM

**Attenuation of Murine Lacrimal Gland Chronic Graft-Versus-Host Disease by Oral Injection of Tranilast**

**Purpose:** Tranilast is a benzoyl isoxazole derivative that is currently used for the treatment of chronic ocular allergy and has been used for the first time in Japan and then approved for medical use. Over the last decades, this molecule has been utilized to treat asthma, atopic dermatitis and keloid. We have shown a significant role of fibroblasts in cGVHD induced pathogenic fibrosis including the epithelial mesenchymal transition (EMT) in lacrimal gland affected by cGVHD. Using cell-based screening methods, tranilast was found to have a potential to suppress EMT in vitro. The purpose of this study is to investigate whether tranilast is capable of mitigating inflammation, fibrosis and oxidative stress in lacrimal gland disorders by cGVHD.

**Methods:** Whole bone marrow transplantation (BMT) was conducted in order to obtain a mouse model of cGVHD. In the case where the donors are B10.D2 mice and the recipients are BALB/c mice, it is allogeneic transplantation and furnishes a mouse model of cGVHD (Zhang, Y. et al J Immunol. 2002). The allogeneic BMT recipients were treated with tranilast or the solvent-vehicle by oral or intraperitoneal injection. We performed (1) histological investigation, (2) immunohistochemical examination, (3) quantitative PCR (qPCR), (4) immunoblot assays and (5) electron microscopic analysis to explore whether oral or intraperitoneal injection of tranilast could mitigate cGVHD in lacrimal glands.

**Results:** Histopathological assessments and immunofluorescence staining suggest that oral or intraperitoneal injection of tranilast suppressed cGVHD-induced, inflammatory cell infiltration, fibrosis and oxidative stress in lacrimal glands. In addition, the results of immunoblot analysis and qPCR indicate that the protein or mRNA expression of NF-kB, CXCL9, TGF-b, and thioredoxin interaction protein (TXNIP) was vastly lower in the lacrimal glands treated with TL compared with those treated with the solvent-vehicle. As indicated by our data, oral injection of tranilast can repress lacrimal gland cGVHD in a more effective manner than intraperitoneal injection.

**Conclusions:** This study suggests that oral administration of tranilast alleviates inflammation and fibrosis caused by ocular cGVHD and...
The OcuCell model mimics interactions at the ocular surface and has significant potential for the toxicological evaluation of therapeutic, diagnostic and conventional CL.

**Commercial Relationships:** Maud Gorbet, None; Dana Toameh, None; Joyce Zhang, None; Chau-Minh Phan, None; Hendrik Walther, None; Lyndon W. Jones, None

**Support:** Natural Sciences and Engineering Research Council of Canada - Engage and USRA programs

**Program Number:** 4728

**Presentation Time:** 5:15 PM–5:30 PM

**Activity of antimicrobial peptides against Stenotrophomonas, Delftia, Elizabethkingia, and Burkholderia isolated from contact lens related adverse events**

Debaran Dutta1, Larke M. Holmlund1, Mark D. Willcox1. 1Optometry, School of Optometry and Vision Science, CCLR, University of Waterloo, Waterloo, ON, Canada; 2Department of Biology, University of Copenhagen, Copenhagen, Denmark.

**Purpose:** Stenotrophomonas maltophilia, Delftia acidovorans, Elizabethkingia meningoseptica, and Burkholderia cepacia have been associated with corneal infiltrative events during contact lens wear. These bacteria are naturally resistant against many first line antibiotics. The purpose of this study was to determine the activity of the antimicrobial peptide Mel4 in solution and when attached on contact lens surface against these Gram-negative bacteria.

**Methods:** In vitro antimicrobial activity of Mel4 was determined against the four Gram-negative bacteria by investigating growth curves for 24 hours by spectrophotometry following by determination of minimum inhibitory concentration (MIC). The Gram negative bacteria were isolated from cornea or contact lens cases of adverse events during contact lens wear. Mel4 was attached onto contact lenses following 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) coupling and characterized by amino acid analysis. Antimicrobial activity of the coated contact lenses against these bacteria was determined by viable plate count.

**Results:** Mel4 was active against all the bacteria tested. Antimicrobial activity was highest against S. maltophilia (MIC = 31.2 µg/ml), and weaker activity was found against D. acidovorans, E. meningoseptica and B. cepacia (MIC = 500 to 1000 µg/ml). Amino acid analysis revealed that Mel4-coated contact lenses were associated with 93 ± 13 µg of peptide. Following surface attachment the inhibition of adhesion against the four bacteria was 90%, 91%, 77% and 53% respectively (P<0.05).

**Conclusions:** Mel4 has varying antimicrobial activity against the Gram negative bacteria that are naturally resistant to antibiotics. Surface attachment this peptide to contact lenses was able to significantly reduce the attachment of these bacteria.

**Commercial Relationships:** Debaran Dutta; Larke M. Holmlund, None; Mark D. Willcox, US7282214 B2 (P)

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