5984 - 1:00PM
VIP Down-Regulates Inflammatory Cell Infiltration in the Pseudomonas Aeruginosa Infected Cornea by Modulating ECM and Adhesion Molecule Expression

Purpose: Previous studies from our laboratory have provided evidence that vasoactive intestinal peptide (VIP) regulates cytokine/chemokine production and host inflammatory cell function to promote resistance against P. aeruginosa corneal infection in normally susceptible C57BL/6 (B6) mice (J. Immunol., in press 2006). This study tested the hypothesis that VIP also regulates extracellular matrix (ECM) and adhesion molecule expression, thus reducing cell migration/infiltration into the P. aeruginosa-infected cornea of susceptible B6 mice and promotes corneal healing and resistance.

Methods: B6 mice were injected i.p. with recombinant (r) VIP daily from -1 through 5 days p.i. Control mice were similarly injected with PBS. Real-time RT-PCR, ELISA and immunohistochemistry (IHC) were used to assess the effects of rVIP treatment in regulation of ECM and adhesion molecule expression.

Results: Injection of B6 mice with rVIP vs. PBS resulted in disparate expression of adhesion molecules and ECM components as detected by real-time RT-PCR. mRNA analysis revealed that corneas of rVIP-treated mice had significantly increased levels for molecules such as: TGF-β1, ITG-α1/2, MMP-9 and -14, and CD44 at 3 and 5 days p.i.; while others including COL1A1, COL1AS1, ICAM-1, VCAM-1, FICAM-1, MMP-2, -3,-5,-7, -9 and -12, ITG-α/β-3, SEL-L and -P were significantly down-regulated when compared to PBS-treated animals. Protein levels for ECM-1 and VCAM-1 supported mRNA data at similar time points, as detected by ELISA. Selective IHC confirmed the effects of rVIP treatment on reducing corneal expression of both ICAM-1 and LFA-1 at 1 day p.i. when compared to PBS-treated animals.

Conclusions: rVIP treatment down-regulates the production of adhesion molecules integral to the transmigration process of host inflammatory cells (PMN, macrophages) into the infected cornea. This results directly in reduced cellular infiltration, less stromal destruction and better disease outcome.

CR: E.A. Szliter, None; R.P. Barrett, None; Y. Zhang, None; L.D. Hazlett, None.
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5986 - 1:30PM
Inhibition of Pseudomonas Aeruginosa Biofilm Formation by Polysorbate 80, a Common Additive to Eye Drops
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Purpose: Bacterial biofilms are associated with pathogenic infections and antibiotic resistance. The gram negative bacterium Pseudomonas aeruginosa (Pa) is able to form biofilms on a variety of medical implants, including contact lenses. Polysorbate 80 (PS80) is a non ionic detergent found in many medications including eye drops. In this report we describe the effect of PS80 on biofilm formation by Pa.

Methods: All genetic, biochemical and microbiologic assays were performed following previously published protocols. Biofilm assays were performed on different abiotic surfaces. Bacteria were incubated in M63 supplemented with glucose and casamino acids for 24 hours at 37°C. The medium was supplemented with PS80 at concentrations ranging from 0.1% to 0.001%.

Results: The lab strain, PA14, is not able to form biofilms on PVC, glass or contact lenses. PS80 is a non ionic detergent found in many medications including eye drops. However, we report here that PS80 inhibits Pa biofilm formation by Pa.

Conclusions: We demonstrate that PS80 inhibits Pa biofilm formation under a variety of conditions and at concentrations lower than those present in commercial eye drops. The resistance of the transposon mutant and other strains to this effect suggests that it is a genetically controlled process. Previous work has shown that Pa lipases can cleave PS80 at its ester bond. Our data suggest that LipA cleavage of PS80 eliminates its antibiofilm effect. These novel observations suggest that PS80, a compound considered inactive by the pharmaceutical industry and well tolerated on the ocular surface, can exert a significant effect on a bacterial group behavior.

CR: M.E. Zegans, None; C.T. Bramante, None; C.M. Toutain, None.
Support: 5K08 EY013977, U101EY05114-01A2

5987 - 1:45PM
Liposomal C6-Ceramide Inhibits Toll Like Receptor (tlr) - Induced Cxc Chemokine Production by Corneal Epithelial Cells and Tlr - Mediated Corneal Inflammation
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Purpose: Activation of Toll-like receptors (TLR) in the corneal epithelium induces an inflammatory response characterized by neutrophil infiltration and loss of corneal clarity. The purpose of the current study was to determine the effect of liposomal C6-ceramide (Lip-C6) on TLR-induced ketosis.

Methods: Liposomes were prepared containing 30 molar C6-ceramide, and control (ghost) liposomes were made in the absence of ceramide. Human corneal epithelial cells were treated with Lip-C6 or ghost liposomes prior to stimulation with inactivated Staphylococcus aureus (TLR2 agonist), and CXC chemokine production was measured by ELISA. For corneal inflammation, C57BL/6 corneas were abraded and treated with Lip-C6 or ghost liposomes prior to activation with S. aureus or LPS, and neutrophil infiltration and corneal haze were measured.

Results: Lip-C6, but not control liposomes, inhibited production of CXCL1, CXCL5 and CXCL8. Furthermore, topical application of Lip-C6 to mouse corneas significantly inhibited S. aureus and LPS-induced corneal inflammation as measured by neutrophil infiltration to the corneal stroma and development of corneal haze. Despite the reported activity for ceramides, Lip-C6 did not induce apoptosis of corneal epithelial cells in vitro or in vivo, nor did it inhibit corneal wound healing.

Conclusions: Together, these findings demonstrate a novel anti-inflammatory, non-toxic, therapeutic role for liposomally-delivered short-chain C6-ceramide in a model of ocular inflammation and resultant visual impairment.

CR: Y. Sun, Bausch and Lomb, Inc.; T. Fox, None; M. Kester, None; E. Pearlman, Bausch and Lomb, Inc., F.
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5988 - 2:00PM
ddc Inhibits Wild Type and ddc-Resistant Ad5 in the Ad5/NZW Rabbit Ocular Model

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Purpose: There remains an unfulfilled need for an effective antiviral to treat adenovirus (Ad) ocular infections worldwide. However, the emergence of viral resistance to an antiviral might limit its usefulness and alter viral pathogenicity. 2',3'-dideoxycytidine (ddC) is a nucleoside analog that demonstrates potent anti-Ad activity in vitro. We created an Ad5 ddc-resistant mutant in vitro that was 50 fold more resistant to ddC than the parental wild type (wt) virus. The goals of the current study were to 1) determine whether ddC would demonstrate anti-Ad activity in vivo against a wt Ad5, 2) whether in vitro ddC resistance altered in vivo pathogenesis, and 3) whether in vitro ddC resistance could be overcome in vivo.

Methods: 20 NZW rabbits were topically inoculated in both eyes, following corneal scarring, with 1 x 10^7 pfu/eye of Ad5 ATCC wt virus (ddC IC50 = 0.08 µg/ml) or the Ad5 ddc-resistant mutant (ddC IC50 = 4.5 µg/ml). On day 1, the rabbits from each virus group were subdivided into 2 topical treatment groups (n=10/group): 1) 2% ddC; II - Control (saline). The rabbits were treated in both eyes QID for 7 days. All eyes were cultured for virus on days 0, 1, 3, 4, 5, 7, 9, 11, and 14.

Results:

<table>
<thead>
<tr>
<th>Virus/Drug Group</th>
<th>Ad + Culture/Total</th>
<th>Duration of Ad Shedding (Days)</th>
<th>Mean Ad Titers (Days 1-5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad5 wt/ddC</td>
<td>9/10 (90.0%)</td>
<td>0.7 ± 1.8</td>
<td>2.2 ± 9.0 x 10^3</td>
</tr>
<tr>
<td>Ad5 wt/DDC</td>
<td>5/10 (50.0%)</td>
<td>4.9 ± 2.2</td>
<td>9.8 ± 16.8 x 10^3</td>
</tr>
<tr>
<td>Ad5 ddc−res mut/ddC</td>
<td>3/10 (30.0%)</td>
<td>3.9 ± 2.0</td>
<td>1.3 ± 4.3 x 10^3</td>
</tr>
<tr>
<td>Ad5 ddc−res mut/DDC</td>
<td>0/10 (0.0%)</td>
<td>7.9 ± 2.5</td>
<td>3.4 ± 17.8 x 10^3</td>
</tr>
</tbody>
</table>

* p < 0.005 Compared to specific virus Control, † p < 0.001 Compared to Ad5 ddc−res mut/Control.

Conclusions: 2% ddC effectively inhibited Ad replication in the Ad5/NZW rabbit model. In vitro selection for ddc-resistance in the Ad5 parental virus was associated with increased ocular pathogenicity in vivo. In vitro ddC resistance was overcome in vivo by the dose regimen of ddC.


Support: NIH Grant 1R01EB005227, NIH Core Grant EY08098, The Eye & Ear Foundation of Pittsburgh.

5989 - 2:15PM
Enhanced Green Fluorescing Protein HSV-1 Keratitis With Healing Time Correlation in Rabbit Models

E.S. Atwal, M. Kumar, E.D. Varnell, H.E. Kaufman. Ophthalmology, Louisiana State University, New Orleans, LA.

Purpose: Healing time in ocular herpetic keratitis has historically been assumed to correspond with virus activity. In the past, demonstrating the presence of ocular Human Herpes Virus Type 1 (HSV-1) without the use of stains for mild epithelial disruption has been without a solution. Moreover, the ability to observe actual HSV-1 on the epithelium or within the stroma of the in situ cornea has been a frank impossibility. Our purpose was to observe the correlation between the presence of the virus and the defect in the epithelium it produces, and the corneal healing as the virus disappears.

Methods: Animals were conducted according to the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research and the NIH Guidelines on the Care and Use of Animals in Research. New Zealand white rabbits were infected with the KOS/EGFP strain of HSV-1 OD and OS. Animals were inspected on the first day post infection via fluorescent microscopy for viral identification and slit lamp examination with fluorescein and cobalt blue filter to establish clinical disease. Photos obtained from these examinations were analyzed by conversion to grayscale, and subsequent color threshold changes allowed us to compare viral activity to epithelial healing.

Results: Preliminary results indicate that clinical disease correlates directly with viral activity on/within the cornea. Invariably, virus appears days before the first noticeable clinical effect can be distinguished. We were able to discern a similar pattern upon the conclusion of viral activity and the correlating end of clinical disease being separated by a few days.

Conclusions: Our results give substance to previous thoughts on HSV keratitis disease course. It is now evident that viral activity has a strong correlation with epithelial disease in HSV keratitis. This model of herpetic keratitis may permit us to make better decisions of when to cease treatments and possibly avoid cytotoxicity from current treatment regimens.

CR: E.S. Atwal, None; M. Kumar, None; E.D. Varnell, None; H.E. Kaufman, None.

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5990 - 2:30PM
Development of a Ribozyme Gene Therapy Against Herpes Simplex Virus Type 1 (HSV-1) Infection

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Purpose: Herpes keratitis is the most common cause of corneal blindness in the US. We proposed a ribozyme gene therapy approach to eliminate HSV infection by inhibiting the expression of HSV essential genes.

Methods: A hammerhead ribozyme (Rz) was designed targeting mRNA of U, 20, an essential HSV-1 gene. The U20 ribozyme was packaged in an adeno vector, and an adeno virus vector expressing GFP was used as control. The Ad-U20 Rz was used to block viral replication of several HSV-1 strains, including wild-type HSV-1 (17gynplus and KOS), and several drug resistant strains (PA-A5, ktlTRZ1, and AGCR4) in tissue culture. The Ad-U20 Rz was tested in vivo in a mouse HSV-encephalitis model. Three groups including PBS, Ad-GFP, and Ad-U20 ribozyme treatments (10 Swiss mice each) were included. Ad-U20 Rz, PBS control or Ad-GFP were injected sub-epidermally and applied topically in rear footpads. HSV-1 infection was conducted right after at a dose of 10^7 pfu (20 fold >LDso). In a separate experiment, animals from either treatment (GFP or ribozyme) were sacrificed and feet, dorsal root ganglion (DRG), and spinal cord were harvested. Viral DNA levels in each tissue were measured using real-time PCR.

Results: U20 Rz significantly inhibited viral replication of all the HSV-1 strains tested including three drug resistant strains, up to 99%. Ad-U20 ribozyme treatment consistently led to a 90% protection for mice from lethal HSV-1 infection, while survival rate in PBS group was 45% and survival in the Ad-GFP group was 40%. Levels of viral DNA in feet, DRG, and spinal cord from animals treated with U20 ribozyme were less than viral DNA levels in tissues from Ad-GFP treated animals.

Conclusions: This study suggests that ribozymes targeting essential herpes genes of the late kinetic class may be a new therapeutic strategy for inhibiting HSV infection. It is possible that knocking down other essential late genes of HSV-1 may provide similar effect. A combination of ribozymes targeting different essential HSV-1 genes might provide synergistic effect and prevent the development of HSV mutants that escape therapy with a single ribozyme. Delivery approach for ribozymes into cornea to prevent HSK is currently under investigation.

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