OK-0301, a Novel Ribonuclease, Demonstrates Antiviral Activity against Adenovirus in the Ad5/NZW Rabbit Ocular Model.  
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Introduction

Adenovirus (Ad) ocular infections (epidemic keratoconjunctivitis [EKC], follicular conjunctivitis, and pharyngeal conjunctival fever) are the most common viral infections worldwide. At present there is no FDA approved antiviral for the treatment of these infections. A novel approach to antiviral development is the use of ribonucleases, which are enzymes that degrade RNA. OK-0301 is a novel ribonuclease that can enter host cells and preferentially degrade viral RNA leading to an inhibition of protein synthesis. It has been previously shown to have antiviral activity against HIV.

Methods

Experimental Drugs – 25 µM and 2.5 µM OK-0301 (OKG) were prepared in IV saline from 1 mg vials of stock drug provided by Okogen. 0.5% Cidofovir (CDV) was prepared in IV saline from the 7.5% injectable form of cidofovir (Cidofovir Injection, [Heritage Pharmaceuticals Inc., Eatontown, NJ]) and served as the positive antiviral control. IV saline (0.9% Sodium Chloride Injection USP [Baxter Healthcare Corp., Deerfield, IL]) served as the negative control (CON).

Virus and Cells – A clinical ocular isolate of adenovirus type 5 (Ad5) was used in the current study. A549 human lung carcinoma cells were used to prepare the virus stock and for the determination of viral titers.

Animals – 1.1 – 1.4 kg female New Zealand White rabbits were obtained from Charles River Oakwood rabbitry. All animal studies conformed to the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research. University of Pittsburgh IACUC approval was obtained and institutional and federal guidelines regarding animal experimentation were followed.

Antiviral Efficacy Study Experimental Design – This study was performed using a total of 38 rabbits. Following appropriate systemic and topical anesthesia, NZW rabbits were inoculated with 50 µl (1.5 x 10⁶ PFU/eye) of Ad5 in both eyes after 12 cross-hatched strokes of a #25 sterile needle. Twenty-four hours later, rabbits were randomly assigned to one of four topical treatment groups:

1) 25 µM OKG  8X/day x 9 Days (n = 9)  
2) 2.5 µM OKG  8X/day x 9 Days (n = 10)  
3) Saline  8X/day x 9 Days (n = 10)  
4) 0.3% CDV  2X/day x 7 Days (n = 9)

Rabbits were treated topically in both eyes according to the above treatment regimens. Ocular swabbing to recover adenovirus from the tear film and corneal and conjunctival surfaces was performed on days 0, 1, 3, 4, 5, 7, 9, 11, and 13 after inoculation and frozen at -80°C pending plaque assay.

Determination of Ocular Viral Titers (Plaque Assay) – The ocular samples to be titrated were thawed, diluted, and inoculated onto A549 cell monolayers. After 7 days incubation, the cells were stained with 0.5% gentian violet, and the number of plaques counted. The viral titers were then calculated, and expressed as plaque forming units per milliliter (PFU/ml).

Statistical Analysis – Ocular titer data was analyzed using Kruskal-Wallis ANOVA with Duncan’s multiple comparisons and Fisher’s Exact Test (True Epistat). Significance was established at the p < 0.05 confidence level.

Results

Figure 1 demonstrates the number of Ad5 Positive Cultures per Total over the course of the study. Significant differences (P < 0.05) were demonstrated among the groups: 25 µM OKG < CDV < 2.5 µM OKG < CON.

Figure 2 demonstrates the median Duration of Shedding. Significant differences (P < 0.05) were demonstrated among the groups: 25 µM OKG < 2.5 µM OKG < CON.

Figure 3 demonstrates the median Ocular Titers during the course of the study. Significant differences (P < 0.05) were demonstrated among the groups: 25 µM OKG < CDV < 2.5 µM OKG < CON.

Conclusions

1. 25 µM OKG-0301 and 2.5 µM OKG-0301 demonstrated significant antiviral efficacy compared with the saline control in the Ad5/NZW rabbit ocular model.

2. The antiviral efficacy of the 25 µM OKG-0301 group was similar to that of the positive antiviral control, 0.5% cidofovir.

3. OKG-0301 appears to be a promising candidate for a topical antiviral for adenoviral ocular infections and further development is indicated.

References


3. Functional Support

Okogen, Inc.; NIH Core Grant EY08098