Abstract

Adenoviruses are non-enveloped, icosahedral viruses containing a DNA genome. Adenovirus D1327 is a serotype known to cause upper respiratory infection. Studies were done preparatory to developing an adenovirus mouse model to determine the antiviral sensitivity of adenovirus strain D1327 before putting it into mice. The use of cell lines A549 (ATCC® CCL-185™), KB (ATCC® CCL-17™) and Hela-Ohio (ATCC, Rockville, MD, USA), were used to test the cytopathic effect (CPE) of the D1327 virus and obtain a titer (lowest concentration of virus effective for infection). These cell lines were used for testing the effectiveness of known drugs in treating the virus in vitro. (Sy3-hydroxy-2-(phosphonomethyl)propyl) (HPMP) drugs and 2’3’-dideoxycytidine (ddC) were selected due to their known inhibition of other adenoviruses previously tested in the Barnard laboratory.

Introduction and Objectives

Adenovirus D1327 is a DNA virus known to cause upper respiratory infection. To develop an animal model for antiviral drug testing, we wanted to first determine the antiviral sensitivity profile of the D1327 strain prior to testing drugs in the animal model infected with the D1327 strain. Octadecyloxypropyl ester 9-(S)-(3-hydroxy-2-phosphonemethoxypropyl)-adenine (ODE-HPMPA), 2’3’-dideoxycytidine (ddC), interferon alfacon 1 (Infergen) and ribavirin were used in varying concentrations to treat infected cells to determine their effectiveness in inhibiting viral propagation and cytotoxicity under normal conditions. Values are expressed in EC50 (50% effective concentration), IC50 (50% inhibitory concentration) and SI (selectivity index). This is done to determine the legitimacy for the use of these drugs in the future mouse model system. The selectivity index will express a direct ratio between the effectiveness of the drug in inhibiting the function of the virus versus causing cytotoxicity. (SI=EC50/IC50)

The main objectives of this project were to:
1. Determine the virulence of D1327
2. Determine the effectiveness of drug treatment in vitro
3. Find effective drug concentrations for preparing tests in vivo

Materials and Methods

- Hela-Ohio cells were cultured with MEM+FBS 10% in 8 75ml T-flasks.
- Once the cells became confluent the media was removed from 4 T-flasks and 4 ml MEM0%+Gentamicin mixed with 40μl was added to 3 of them (one had no virus added to provide a mock flask.
- These flask were shaken for one hour before adding 2 ml MEM0%+Gentamicin and 4 ml MEM+4%FBS
- This was cultured for about 6 days at 37° C and 5% CO2 before Freezing.
- This new pool of virus was labeled V2426 and placed in 0.5 ml vials after dilutions and then serially diluted in MEM by half-log dilutions in triplicate for toxicity in duplicate
- For each cell line, cells were cultured to confluence and cell counts were obtained.
- These cell counts were then used to culture the cells at 2ε4 cells/well of a 96 flat bottom well plate.
- The cells were then cultured until they became confluent within the wells and then infected with a 1:100 dilution of the V2426 pool of virus
- 6 wells were used as a control for cells, 6 wells were used as a control for infected cells.
- Drug concentrations were tested for toxicity in duplicate.
- Drug effectiveness in inhibiting viral function was tested in triplicate.

In vitro antiviral assays: compounds were tested for efficacy in vitro using the virus cytopathic effect (CPE) reduction assays followed by neutral red (NR) uptake assays on the same plate. Compounds were dissolved in appropriate dilutions and then serially diluted in MEM by half-log dilutions in triplicate wells. Test wells were infected with a multiplicity of infection (MOI) of <0.007 CCID50/cell to allow near complete CPE in 3 days time. Test medium used was MEM+2%FBS+50 μg/ml Gentamicin. Toxicity was assayed in duplicate wells without virus. After incubating for 3 days each well was read microscopically for CPE. Media was removed and plates incubated with 0.011% NR for 2 hrs. Free NR was removed and uptaken NR eluted in 50% ethanol in Sorenson citrate buffer for >30min. Absorbance was measured with Opsys MR microplate reader (Dyhex, Chantilly, VA) at 540nm/405nm. Absorbance values and visual CPE expressed as percentages of cell controls and untreated virus controls adjusted for toxicity. EC50 and IC50 calculated by regression analysis.

The diagram below shows how the wells were designated during drug rounds.

The table below was used to determine EC50, IC50 and SI values to determine the effectiveness of the drug treatment.

Conclusions

- ODE-HPMPA was determined to be a poor drug for adenovirus D1327.
- Several other variations of HPMP drugs are possibly more effective treatments.
- HPMP drugs with different natural nucleotide analogs such as HPMPG or HPMPC may be more effective and should be tested.

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