Influenza viruses are classified based on their surface glycoproteins: hemagglutinin and neuraminidase. Hemagglutinin (HA) is responsible for binding to the host cell, while neuraminidase (NA) facilitates escape of newly formed virus particles. These studies determined the NA activity of three subtypes of influenza A virus that differ in their ability to cause disease in mice: influenza A/NWS/33 (H1N1), influenza A/Victoria/3/75 (H3N2), and influenza A/Duck/MN/1525/81 (H5N1). Chemiluminescent quantitation of NA activity in equal amounts of each virus was studied. In the 2007-2008 winter, NA inhibitors were used and studied today. Influenza has shown an incredible success and ability to cause disease. A focus of current chemotherapeutic treatment regimens involve inhibiting the roles of NA in influenza, which are important for its spread. Neuraminidase activity is critical for the virus to release itself from infected cells [1]. This in turn will cause subsequent virus infection. The effects of oseltamivir on viral neuraminidase activity were performed using a commercially available kit (NA-Star® Influenza Neuraminidase Inhibitor Resistance Detection Kit). Applied Biosystems, Foster City, CA) in 96-well solid white microplates following the manufacturer’s instructions [4,6]. Oseltamivir in half-log dilution increments was incubated with virus (as the source of neuraminidase). The amount of virus in each microwell was approximately 500 cell culture infectious doses (CCID₃). Plates were pre-incubated for 20 minutes at 37°C prior to addition of chemiluminescent substrate. Following addition of substrate plates were incubated for 30 minutes at 37°C. The neuraminidase activity was evaluated using a Centro LB 960 luminometer (Berthold Technologies) for 0.5 seconds immediately after addition of NA-Star® accelerator solution. Fifty percent virus-inhibitory concentrations (EC₅₀ values) of viral neuraminidase activity were determined by plotting percent chemiluminescent counts versus log of oseltamivir concentration. NA activity of each virus subtype was determined by completing the assay using only buffer.

Antiviral activity of oseltamivir carbonate, the active form of oseltamivir, was determined in Madin-Darby canine kidney (MDCK) cells. The cells were grown in MEM supplemented with 5% FBS. The assays were completed in 96-well microplates incubated with approximately 50 CCID₃ of virus. Microplates were visually examined after 3 days of infection and then treated for 1 hr with neutral red (0.01%) final concentration to quantify the virus-induced cytopathic effects (CPE). Excess dye was rinsed from cells with PBS. The absorbent dye was eluted from the cells by addition of 0.1 ml of 50% Sorenson’s citrate buffer/50% ethanol to each well. Optical density (OD) measurements were completed on the microtiter plates at 560 nm. OD readings were converted to percent of uninfected control using an Excel spread sheet developed for this purpose. EC₅₀ values were determined by plotting percent CPE versus logₐ of inhibitor concentration.

The virus titers were 1 x 10⁶, 1 x 10⁻⁴, and 1 x 10⁻⁶ PFU/ml for NWS, Victoria, and Duck viruses respectively. Virus titers were equalized using dilutions. The dilutions used were 1:5, 1:90, and 1:90 for NWS, Victoria, and Duck viruses, respectively.

Results

The virus titers were 1 x 10⁶, 1 x 10⁻⁴, and 1 x 10⁻⁶ PFU/ml for NWS, Victoria, and Duck viruses respectively. Virus titers were equalized using dilutions. The dilutions used were 1:5, 1:90, and 1:90 for NWS, Victoria, and Duck viruses, respectively.

Figure 2: Neuraminidase activity of each virus subtype tested at equal virus concentrations.

The virus titers were 1 x 10⁶, 1 x 10⁻⁴, and 1 x 10⁻⁶ PFU/ml for NWS, Victoria, and Duck viruses respectively. Virus titers were equalized using dilutions. The dilutions used were 1:5, 1:90, and 1:90 for NWS, Victoria, and Duck viruses, respectively.

Figure 3: Neuraminidase activity graphed as percent chemiluminescence vs oseltamivir concentration in nM.

Conclusions

These studies conclude that NA subtype one (N1) has a higher NA activity compared to NA subtype two (N2). The Duck virus is most resistant to oseltamivir in the NA-Star® assay used in cell culture studies. These data suggest there is correlation between NA activity and virus resistance to oseltamivir. This research will hopefully lead to better ways of identifying drug-resistant influenza virus strains.

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