ABSTRACT

Aquatic birds are the natural reservoir of influenza virus. To date, 16 influenza subtypes have been identified in wild birds. These studies involved the characterization of influenza A virus obtained from the American Type Culture Collection (ATCC) that had been collected from wild birds. Virus stocks of each influenza virus isolate were prepared and characterized for hemagglutinin (HA) and neuraminidase (NA) activity. Influenza virus HA binds to cellular receptors in the respiratory tract to initiate the infection. Viral NA aids in the release of virus from infected cells. HA activity was determined using red blood cells from different species, and NA activity was measured by enzyme assay. These studies will determine if virus stocks collected from different species of birds and different locations show any differences in HA or NA enzyme assay. These studies will determine if virus isolates from wild birds have similar drug sensitivity profiles to human clinical isolates.

INTRODUCTION

The influenza virus is of great interest because of its capacity to pass easily from one individual to another. One proposed cause of this is neuraminidase (NA) activity. NA is an enzyme that is attached to the membrane of the influenza virus. The activity of NA is critical for the virus to release itself from infected cells [1]. This in turn will cause subsequent virus spread. NA has also been proposed to aid in the entry of the virus into target cells [2]. The roles of NA in influenza is important for its success and ability to cause disease. This research was to determine if NA activity differed among strains collected at different times and regions from varying species of wild birds. A focus of current chemotherapeutic treatment regimen involves inhibiting the activity of the NA enzyme. These antiviral drugs are called neuraminidase inhibitors. Influenza has shown an incredible increase of resistance to oseltamivir, a type of NA inhibitor. In the 2007 - 2008 winter, the virus tested positive for resistance 11% of the time from throat swabs of patients with the most common influenza subtype [3]. This research looked for a pattern in NA activity between isolates collected from wild type birds to determine if there is correlation between Influenza virus from wild birds compared to Influenza virus found in humans.

METHODS

Viral neuraminidase inhibition assay

The effect of oseltamivir on viral neuraminidase activity was performed using a commercially available kit (NA-Star® Flu Influenza Neuraminidase Inhibitor Resistance Detection Kit, Applied Biosystems, Foster City, CA) in 96-well solid white microplates following the manufacturer’s instructions. Oseltamivir in half-log dilution increments was incubated with virus (as the source of neuraminidase). The amount of virus in each was approximately 500 cell culture infectious doses (CCID50). Following addition of substrate plates were incubated for 10 min at 37°C. The neuraminidase activity was evaluated using a Centro LB 960 luminometer (Berthold Technologies) for 0.5 sec immediately after addition of NA-Star® accelerator solution.

RESULTS

1. Neuraminidase (NA) assay using the NA Star kit (enzyme assay) on 22 viruses from wild birds.
2. Hemagglutinin (HA) assay on all the virus isolates using red blood cells (RBCs).
3. Cell-culture based (96-well plate) antiviral assay evaluating sensitivity to oseltamivir and amantidine.

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