Effect of ultraviolet radiation on West Nile Virus

Introduction

WN fever is a mosquito-borne flavivirus infection that is transmitted to vertebrates primarily by various species of *Culex* mosquitoes. Like other members of the Japanese encephalitis (JE) antigenic complex of viruses, including JE, St. Louis encephalitis (SLE), and Murray Valley encephalitis viruses, WN virus is maintained in a natural cycle between arthropod vectors and birds. The virus was first isolated from a febrile human in the West Nile district of Uganda in 1937. It was soon recognized as one of the most widely distributed flaviviruses, with its geographic range including Africa, the Middle East, western Asia, Europe, and Australia. Clinically, WN fever in humans is a self-limited acute febrile illness accompanied by headache, myalgia, polyarthropathy, rash, and lymphadenopathy. Rarely, though, acute hepatitis or pancreatitis has been reported, and cases in elderly patients are sometimes complicated by encephalitis or meningitis. Currently, no human or veterinary vaccine is available to prevent WN virus infection, and mosquito control is the only practical strategy to combat the spread of disease.

The current geographic distribution of WN virus includes 27 states. The presence of the virus was first detected this year (2001) in two Louisiana parishes, Jefferson and Vermillion. The spillover of WN virus to humans and horses did occur in 2001 in Louisiana with one human case and 4 horses diagnosed with WN virus. This trend is expected to continue with more cases of encephalitis, similar to what has occurred in other states when the viral activity increases with amplification. Louisiana has a long history of vector-borne disease transmission because of the geographical sub-tropical location of the state and in recent years has experienced an increase in the number of arbovirus-related encephalitis cases in both humans and horses. The state is located in a unique biotic and geographic area of the Mississippi delta with factors such as climate, blood-feed arthropods, and vertebrate hosts, that allows for the complex life cycle of zoonotic arboviral encephalitides including WN virus, St. Louis encephalitis virus and Eastern equine encephalitis virus surviving in this area. In the United States, 9 genera and over 20 species of mosquitoes, many of which are found in Louisiana, have been either found naturally infected with WN virus or shown to become infected under laboratory conditions. The high rate of severe neurologic illness and death in humans, horses, and birds are unprecedented and unexplained. West Nile (WN) virus was first detected in the United States in New York City and By the year 2002 the geographic distribution of WNV includes 25 states including Louisiana. Currently there is no human vaccine available to prevent WN virus infection and serological procedures for diagnosis of the infections are complicated because of cross-reaction with heterologous flaviviruses.

The Virus. WNV is a member of family *Flaviviridae*, and the genus *Flavivirus*. Based on relatedness of antigenic determinants WNV is a member of Japanese Encephalitis (JE) virus serocomplex. The group includes JE found in Asia; St. Louis encephalitis (SLE) found in America, Kunjin (subtype of WN) and Murray Valley encephalitis virus found in Australia. All members of the JE complex are associated with human encephalitis. West Nile virus is a single-stranded positive-sense RNA of approximately 12,000 nucleotides. The genomic RNA contains a single long ORF encoded polyprotein that is translated and co and post translationally processed by viral and cellular proteases into 3 structural and 7 nonstructural proteins. The three structural proteins include the capsid, two integral membrane glycoproteins, a premembrane (prM) and E glycoprotein. The seven nonstructural proteins include the NS1, NS2, NS2b NS3, NS4a, NS4b, and NS5. The genus includes more than 68 members and are grouped according to a serological relatedness based on viral hemagglutinin and neutralizing antibodies of the E glycoprotein. The E glycoprotein has been used to provide evidence for the presence of group, serocomplex and type-specific determinants. This antigenic classification conforms to major biological and epidemiological characteristics of the flaviviruses. Glycoprotein E-specific monoclonal antibodies have also been used to confirm the relatedness of the group, serocomplex and type specific antigenic determinants. Based on nucleic acid sequence information and the amino acid sequence of the E glycoprotein, this glycoprotein demonstrates a perfect match of the grouping of the flavivirus serocomplexes.

The Louisiana WN strain (isolated from a blue jay 2001) has been cultured in Vero cells and the complete genome sequenced by the Division of Biotechnology and Molecular Medicine, LSU School of Veterinary Medicine (BIOMMED) directed by Dr. K. G. Kousoulas. When comparing the sequence of the WN virus Louisiana 2001 strain (WN-LA01), to the WN virus New York 1999 strain (WN-NY99), 27 base changes in the region that includes nucleotide position 969-10085 were noted. There is one mutation at position 5758 in which an amino acid change occurs with an A substituting a G. This mutation is in a nonstructural gene that encodes a polymerase enzyme.

Effect of UV radiation on viruses. UV has long been known to inactivate viruses while preserving their ability to be used as antigens in the preparation of vaccines. Viral genomes are susceptible to UV-induced damage, which can cause lethal breaks and mutations within viral genomes. Thus, viruses can be potentially killed by damage to their nucleic acids while, at the same time, leaving antigenic surface components (proteins, glycoproteins, and/or fatty acids) intact. Killed viruses can be recognized by the immune system generating humoral and cellular immunity against the virus, which can protect the host against future infections. Preliminary investigations with coronaviruses indicated that a exposure to UV radiation emitted by the Energex Hemo-Modulator (Energex Systems, Inc., Emerson NJ). inactivated coronaviruses by more than one-and-a half logs. Coronaviruses are single stranded (+ sense) RNA of approximately 30 kb, while West Nile Virus is approximately 12 Kb. Thus, it is expected that UV

radiation should have similar effects against West Nile Virus, perhaps at lower inactivation efficiency due to the smallest size of its genome in comparison to the coronaviruses.

STUDY PROTOCOL

Purpose

This study is conducted to determine the extent of inactivation by ultraviolet radiation of West Nile virus, The device utilized in this study is known as the Energex Hemo-Modulator (Energex Systems, Inc., Emerson NJ) a blood irradiator that produces Ultraviolet energy ranging from 200nm - 400nm.

Design

Different amounts of West Nileviral loads in buffered growth medium and horse blood will be treated with ultraviolet radiation and the infectious titers will be determined before and after irradiation to estimate the extent of inactivation.

Study protocol

1. West Nile isolate (WN-LA01) at titers of 7 and 5 logs will be spiked into 50 ml samples of buffered growth medium (DMEM+10 fetal calf serum) at a hundred-fold dilution to obtain a final titer of 5 and 3 logs.
2. The spiked samples will be treated under standard UV radiation protocol and they will be collected in 3 equal fractions after treatment.
3. WN-LA01 titers will be determined before treatment and after UV irradiation in each of the 3 fractions collected. As a control, WN-LA01 titers will be determined in sham-irradiated samples.
4. WN-LA01 Infectious titers will be assessed by plaque formation on human African monkey kidney cells (Vero) using 10-fold serial dilutions.
5. WNV-LA01 infectious titers will be also assessed for confirmatory purposes by indirect immunofluorescence assays using monospecific antibodies against WN virus.

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West Nile vs. Hemo-Modulator