

# Therapy of West Nile Virus Infection

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There was an incursion of West Nile virus (WNV) into the New York City area of the United States in 1999<sup>1</sup> and subsequently there has been rapid geographic expansion through the North American continent with human cases occurring in Canada during both the 2002<sup>2</sup> and 2003 seasons. Clearly, WNV infection has now become endemic in wild birds and there will continue to be a threat for transmission to humans via mosquitoes during the summer and fall months on a yearly basis. Most WNV infections are subclinical or cause only a mild systemic illness without neurological manifestations. In a minority of individuals WNV infection has been recognized to be associated with a variety of neurological syndromes in addition to the relatively common presentations with encephalitis and aseptic meningitis. Patients may develop asymmetrical flaccid muscle weakness, frequently with respiratory muscle involvement, due to myelitis (poliomyelitis-like) with infection of spinal cord motor neurons and/or involvement of ventral nerve roots.<sup>3-5</sup> Optic neuritis<sup>6</sup> and a variety of movement disorders,<sup>7,8</sup> cerebellar syndromes, and other neurological problems have been associated with WNV infection. In this issue of the journal, Sayao et al<sup>9</sup> have made the first report of WNV infection presenting with the opsoclonus-myoclonus syndrome.

Therapy of patients with WNV encephalitis or encephalomyelitis is a challenging problem facing neurologists and infectious disease specialists. Therapy of flavivirus infections has been only supportive since there are no available antiviral or other drugs with proven efficacy. Ideally, studies of potential therapies should first be assessed in good animal models with neurological disease before they are used for the treatment of human disease. The lack of data from animal studies and the abundance of human cases during 2002 and 2003 have led to the use of therapeutic agents without the benefit of this important information. The most promising potential treatment options that are available at the present time for human therapy include ribavirin, interferon- $\alpha$ , anti-WNV immunoglobulin, and gene-targeted technologies. Each of these therapies will be discussed individually.

## RIBAVIRIN

Ribavirin is a synthetic nucleoside analogue and a RNA mutagen<sup>10,11</sup> with *in vitro* activity against several viruses. Ribavirin also has immunomodulatory properties that may, in part, account for its antiviral properties *in vivo*.<sup>12</sup> Ribavirin has *in vitro* activity against WNV infection, although efficacy has not yet been demonstrated in an animal model. Ribavirin has antiviral activity at high doses *in vitro*, inhibiting viral replication and cytopathogenicity, with a measured ED<sub>50</sub> of 60  $\mu$ M (and ED<sub>90</sub> of 190  $\mu$ M) for reduction of extracellular viral RNA in human oligodendroglial cells.<sup>13</sup> Lack of efficacy in central nervous system (CNS) viral infections may be attributed to

failure of the drug to adequately cross the blood-brain barrier. Rapid uptake into cerebrospinal fluid (CSF) was not observed in rats and rhesus monkeys.<sup>14</sup> Ribavirin has low lipid solubility with a partition coefficient of -2.06, resulting in ineffective passage through lipid membranes.<sup>15</sup> However, significant levels of ribavirin were observed in CSF after several weeks of oral ribavirin therapy in patients with AIDS and AIDS-related complex.<sup>16</sup> Ribavirin is typically administered intravenously with both loading and maintenance doses. Administration of ribavirin intraventricularly via an Ommaya reservoir would be a therapeutic option for therapy of WNV infection at the present time, and this approach has recently been shown to be safe and well-tolerated for the treatment of subacute sclerosing panencephalitis (SSPE).<sup>17</sup> No clinical trials of ribavirin in WNV infection have been reported. Thirty-seven patients received ribavirin in an outbreak in Israel in 2000 and high mortality (41%) was noted in this group, but details of the therapy were not reported.<sup>18</sup>

## INTERFERON- $\alpha$

Interferon- $\alpha$  is a natural immunoregulatory protein and an immunotherapeutic drug for viral and neoplastic diseases.<sup>19</sup> Interferons provide a first line of defense against viral infections by generating an intracellular environment that restricts viral replication. Interferon- $\alpha$  interacts with cells of the innate immune system and participates in the transition into an effective adaptive-immune response, including antigen presentation for activation of cytotoxic T-cell responses.<sup>19</sup> Interferon- $\alpha$  may also act synergistically with antibody, which has been demonstrated in Sindbis virus infection.<sup>20</sup> Anderson and Rahal<sup>21</sup> have demonstrated *in vitro* inhibition of WNV-induced cytotoxicity with concentrations of interferon- $\alpha$  2b of 5.9 U/mL or higher when added 1.5 hours after infection. Surprisingly, interferon- $\alpha$  2b has not yet been evaluated in an animal model of WNV infection.<sup>22</sup> A double-blind, placebo-controlled clinical trial in Vietnam failed to find a difference in mortality or functional outcome with intramuscular administration of interferon- $\alpha$  2a (10 million units/m<sup>2</sup> body surface area daily for seven days) for treatment of Japanese encephalitis.<sup>23</sup> Intravenous administration of interferon- $\alpha$  results in only low levels in the CSF,<sup>24</sup> and the systemic administration of a low dosage (vs. over 50 million U/m<sup>2</sup> used for SSPE and rabies encephalitis), which was initiated relatively late in the clinical course, may explain the lack of a therapeutic benefit. Intraventricular administration of interferon- $\alpha$  and/or interferon- $\alpha$  has been used for the treatment of SSPE.<sup>17,25</sup> Therapy with interferon- $\alpha$  has been shown to be effective in a mouse model in preventing lethal infection with St. Louis encephalitis virus at around the time of exposure to the virus or shortly thereafter (reducing mortality by up to 70%).<sup>26</sup> However, late treatment was not effective in this model.

In this issue of the journal, Sayao et al<sup>9</sup> describe three patients

treated with open label interferon-  $\beta$ . Although improvement of these patients gives us optimism that this therapy may be effective, only data from properly constructed clinical trials will give us useful information about the efficacy and safety of a therapeutic agent. A randomized unblinded clinical trial of interferon-  $\beta$  (Principal Investigator James Rahal, New York Hospital Queens, New York) has been initiated in the United States (<http://nyhq.org/posting/rahal.html>). Random enrollment of 40 patients is planned to either treatment with interferon-  $\beta$  (3 million units intravenously followed by 3 million units subcutaneously after 12 hours and then daily for 14 days) or to no treatment. Initiation of this study in 2002 was reported widely by the news media, including CNN.<sup>27</sup>

#### ANTI-WNV IMMUNOGLOBULIN

There is evidence that WNV may be susceptible to antibody-mediated immune responses. In mice inoculated intraperitoneally with WNV, Camenga and co-workers<sup>28</sup> gave cyclophosphamide to suppress the humoral and T cell-mediated arms of the immune response. The mice were then reconstituted with either immune serum or syngeneic spleen cells at various time points. Eighty-two percent of mice could be rescued at day 5 or 6 (virus detectable in the brain at day 6) with immune serum (vs. survival in 3% of controls), but not with syngeneic spleen cells. However, administration of immune serum at day 8 or 10 resulted in only 22% survival. In a model of WNV encephalitis in Syrian golden hamsters using intraperitoneal inoculation, viremia was detected for the first seven days after inoculation and neuronal degeneration was first noted by day 5 and death occurred in 50-70% of animals between seven and 14 days after inoculation.<sup>29</sup> Antibody was protective when given 24 hours before inoculation in another study using the same model.<sup>30</sup> However, if antibody administration was delayed until 48 hours after inoculation, then no survival effect was seen.<sup>22</sup> Ben-Nathan and co-workers<sup>31</sup> used an IgG preparation from human Israeli blood donors (anti-WNV antibody titer of 1:1600 by ELISA and of  $>1:80$  by plaque-reduction test) and mouse anti-WNV hyperimmune serum (ELISA titer 1:3200), which were administered intraperitoneally one day before and on day 1 and 3 after intraperitoneal inoculation of WNV. The human IgG preparation was also given in various regimens after administration of WNV, but always included a dose at day 3 or earlier after WNV administration. In this model viral invasion of the brain occurred about three days after inoculation of WNV. Survival of mice dropped to 50% when immunoglobulin was administered on day 3 and 4. It is unclear if the therapeutic benefit was limited to administration of immunoglobulin during the viremic phase of the infection. Recently, Diamond and co-workers have reported experimental infection in adult C57BL/6 mice after footpad inoculation with the New York strain of WNV, and found viral spread into the brain on four to five days postinoculation.<sup>32,33</sup> Administration of human gamma globulin containing anti-WNV antibodies reduced mortality when given as late as five days after viral inoculation. However, in this model clinical neurological disease did not develop until seven to 10 days postinoculation.<sup>32</sup> Hence, administration of immunoglobulin only had a therapeutic effect when it was given at time points *prior* to the development of clinical neurological disease. In summary, there is not yet any

experimental evidence that therapy with immunoglobulin will improve survival or neurological outcome of experimental animals when this therapy is initiated after the development of clinical neurological disease. Clearly, this is the important challenge in developing therapy for human disease.

In a model of experimental encephalitis in mice using infection with Sindbis virus, adoptive transfer of immunoglobulin was shown to result in clearance of infectious virus and viral RNA from the CNS.<sup>34,35</sup> Furthermore, it was demonstrated that antiviral monoclonal antibodies could restrict viral gene expression in infected neurons. Administration of rabies virus-neutralizing monoclonal antibodies (e.g., monoclonal antibody 1112-1) has also been shown to clear rabies virus infection from the CNS in a rodent model when administered before the onset of clinical signs, resulting in survival of experimentally-infected rats.<sup>36</sup> This monoclonal antibody inhibited viral spread from cell to cell and restricted rabies virus RNA transcription. These experimental studies in animals indicate optimism that antibodies may potentially facilitate viral clearance from the CNS and promote resolution of neurologic injury. However, this effect has not yet been demonstrated in any flavivirus infection in humans or experimental animals. If there is an immunopathological component to the neurological disease, then administration of immunoglobulin could aggravate the clinical disease resulting in increased morbidity and mortality. Another concern is that progressive neuronal damage might occur after clearance of infectious virus, which has been recently shown by Kimura and Griffin<sup>37</sup> after passive transfer of immune serum in experimental neuroadapted Sindbis virus encephalomyelitis in mice. Delayed or chronic neurological disease has also been observed after passive transfer of antibody in experimental measles virus infection<sup>38</sup> and Semliki Forest virus infection<sup>39</sup> in mice. For these reasons, studies should ideally be performed in animal models before therapies are given to patients or clinical trials are initiated.

There have been two reports of possible benefit of administration of intravenous immunoglobulin (IVIG) in immunocompromised patients. The first report was a 70-year-old female with a 12 year history of chronic lymphocytic leukemia.<sup>40</sup> She progressed to coma within three days after the onset of her illness due to WNV infection and she had a CSF pleocytosis and IgM against WNV in both serum and CSF. She was treated with IVIG (Omr-IgG-am, Omrix Biopharmaceutical Ltd, Tel Hashomer, Israel) obtained from Israeli donors, 0.4g/kg, and her level of consciousness returned to normal over five days. Because of the presence of endemic WNV infection in Israel, immunoglobulin preparations pooled from donors in Israel contain high titers of antibodies against WNV. A 42-year-old lung transplant recipient with serologically confirmed WNV encephalitis and deteriorating level of consciousness (described as confused and became more obtunded on the next day) received the same treatment and showed rapid improvement over 24 hours and his clinical disease resolved over 48 hours.<sup>41</sup> Another 55-year-old male with chronic lymphocytic leukemia and recent chemotherapy developed generalized muscle weakness and memory impairment, which progressed to obtundation.<sup>42</sup> There was serological confirmation of WNV infection and PCR on CSF was positive for WNV. He received five 0.5g/kg doses of Israeli IVIG (Omr-IgG-am) over a six-day

period. He had persistent coma and magnetic resonance imaging showed progressive brain lesions, and he died 32 days into his illness. Hence, no beneficial effect of the therapy was demonstrated in this case.

The Collaborative Antiviral Study Group (Principal Investigator Richard Whitley, University of Alabama, Birmingham, AL) has initiated a phase I/II randomized, placebo-controlled trial to assess the safety and efficacy of intravenous IgG containing high anti-WNV titers in patients either with or at high risk for progression to WNV encephalomyelitis (see <http://www.casg.uab.edu/> for a list of active sites in the United States). In this study hospitalized adult patients will be randomized to receive Omr-IgG-am<sup>TM</sup>, an immunoglobulin containing no anti-WNV antibodies, or saline placebo. Initiation of this study in 2003 was also announced by the news media, including USA Today.<sup>43</sup>

### GENE-TARGETED TECHNOLOGIES

Antisense compounds can be synthesized that recognize and bind to target viral gene sequences, resulting in a block in mRNA translation and protein synthesis. Recently, antisense technology using phosphorodiamidate morpholine oligomers has been applied to a number of emerging viral diseases, including infections caused by calcivirus<sup>44</sup> and WNV. AVI Biopharma (Corvallis, Oregon) has developed an antisense compound, AVI-4020, targeting WNV and has initiated phase I/II human clinical studies. During 2003 a total of 10 patients were enrolled in Colorado and AVI Biopharma reported no safety concerns (<http://www.avibio.com/>). Genetic-targeted technologies, including antisense compounds, hold promise for the future in the treatment of WNV and other viral infections.

### COMBINATION THERAPIES

It is possible that a combination of specific therapies may be more effective than single agents and this approach is being used for other viral diseases. For example, ribavirin and interferon- provide a clinically synergistic effect in the treatment of chronic hepatitis C infection.<sup>10,45,46</sup> Combination therapy with intraventricular interferon- / and ribavirin has also been used for the treatment of SSPE.<sup>17,25</sup> No reports have yet been published using combination therapy in WNV infection. Unfortunately, we do not yet have any therapies that are known to be effective for the treatment of WNV neurological disease. Hopefully, the results of studies in both animal models and in human clinical trials will soon become available.

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