Treatment of Intracranial Dengue Virus Infections in Mice with a Lipophilic Derivative of Ribavirin

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Rimantadine, ribavirin, and 6-mercaptop-9-(tetrahydro-2-furyl)purine, administered intraperitoneally every 8 h for 7 days starting minutes after virus challenge, had no effect on survival and mean survival time of BALB/c mice inoculated intracranially with dengue virus type 2. In contrast, intraperitoneal treatment with ribavirin 2',3',5'-triacetate, a lipophilic analog of ribavirin, effected significant increases in both mean survival time and survival rate, suggesting that ribavirin 2',3',5'-triacetate may be superior to rabavirin for treatment of viral diseases of the brain.

Previous studies in this and other laboratories have identified several antiviral compounds capable of inhibiting the replication of dengue viruses in a variety of tissue culture systems (6–8, 11). The anti-influenza agents amantadine and rimantadine effectively reduce the growth of all four types of dengue viruses in LLC-MK2 cells and in human peripheral blood leukocytes (6, 7). Based on minimal inhibitory concentration analyses, rimantadine appears to be more effective than amantadine in limiting the replication of dengue viruses in vitro (7). Ribavirin, a broad-spectrum antiviral agent, suppresses the growth of dengue viruses in LLC-MK2 cells but has no effect on dengue virus growth in human peripheral blood leukocytes (8). In addition, it has been shown that ribavirin plus 6-mercaptop-9-(tetrahydro-2-furyl)purine (6-MPTF) can significantly inhibit the replication of dengue viruses in human peripheral blood leukocytes at drug concentrations well below cytotoxic levels (8).

In this present study, rimantadine, ribavirin, ribavirin 2',3',5'-triacetate (RTA), and 6-MPTF have been evaluated for their efficacies against dengue virus infections in vivo through the use of a murine model system. For experimental evaluations of antiviral compounds, 4- to 6-week-old female BALB/c mice weighing 15 to 20 g each were used throughout these studies. Dengue virus type 2, strain Trinidad 1751, was passaged 27 times in suckling mice and recovered from brains, titrated in LLC-MK2 cells (5), and stored at 4.0 × 10⁷ PFU/ml at −70°C. Viruses were diluted in sterile 0.85% saline, and 10 50% lethal doses of dengue virus type 2 (ca. 6.0 × 10⁴ PFU per 50% lethal dose) in a total volume of 0.03 ml were inoculated intracranially into the BALB/c mice. All drugs were obtained as dry powders, dissolved in sterile 0.85% NaCl, filtered, and stored at 4°C throughout the course of each experiment. Rimantadine hydrochloride (α-methyl-1-adamantanemethylamine hydrochloride) was obtained from E. I. du Pont de Nemours & Co., Wilmington, Del. Ribavirin (1β-β-β-ribofuranosyl)-1,2,4-triazole-3-carboxamide) and RTA were obtained from Viratek, Inc., Covina, Calif. 6-MPTF was obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Silver Spring, Md.

Immediately after virus inoculations, mice were treated intraperitoneally (i.p.) with either 0.5 ml of 0.85% saline (control) or 0.5 ml of antiviral agents dissolved in 0.85% saline. Drug or saline treatments were repeated every 8 h for a period of 7 days. The duration of all experiments was 21 days. Mice were monitored daily for quantitation analyses of mean survival and percent survival. Student’s t test was used to evaluate the significance of observed differences between experimental and control groups in the mean survival time analyses. The chi-square test with the Yates correction was used for percent survival analyses.

Treatment of dengue virus-infected BALB/c mice with rimantadine was not effective in altering the course of infection, as monitored by mean survival time and percent survival rates (group A, Table 1). Similarly, i.p. treatment of

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TABLE 1. Effect of antiviral chemotherapy against dengue virus type 2 infections of BALB/c mice

<table>
<thead>
<tr>
<th>Expt group</th>
<th>Compound (no. of mice)</th>
<th>% Survival</th>
<th>Mean (± SD) survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control (26)</td>
<td>11.5</td>
<td>11.16 ± 1.98</td>
</tr>
<tr>
<td></td>
<td>Rimantadine (19)</td>
<td>10.5</td>
<td>11.35 ± 1.66</td>
</tr>
<tr>
<td>B</td>
<td>Control (24)</td>
<td>12.5</td>
<td>10.44 ± 1.67</td>
</tr>
<tr>
<td></td>
<td>Ribavirin (12)</td>
<td>16.7</td>
<td>10.00 ± 1.41</td>
</tr>
<tr>
<td></td>
<td>RTA (18)</td>
<td>44.4</td>
<td>16.91 ± 3.18</td>
</tr>
<tr>
<td>C</td>
<td>Control (42)</td>
<td>14.6</td>
<td>10.09 ± 1.46</td>
</tr>
<tr>
<td></td>
<td>RTA (30)</td>
<td>38.9</td>
<td>15.11 ± 2.98</td>
</tr>
<tr>
<td></td>
<td>6-MPTF (12)</td>
<td>0</td>
<td>8.50 ± 1.37</td>
</tr>
<tr>
<td></td>
<td>RTA + 6-MPTF (12)</td>
<td>0</td>
<td>8.00 ± 2.39</td>
</tr>
</tbody>
</table>

a Experimental methods are described in the text.  
b Rimantadine was administered at a dose of 50 mg/kg of body weight.  
c Ribavirin and RTA were administered at a dose of 50 mg/kg of body weight.  
d 6-MPTF was administered at a dose of 10 mg/kg of body weight, which is well below toxic levels for mice.

dengue virus-infected mice with ribavirin did not significantly affect the course of infection (group B, Table 1). RTA is a lipophilic analog of ribavirin (2). When RTA was administered i.p. three times daily to dengue virus-infected BALB/c mice for a period of 7 days post-virus inoculation, it significantly increased the mean survival time (P < 0.01) and percent survival (P < 0.05) as compared with those of saline-treated control animals (groups B and C, Table 1). Previous studies in this laboratory have demonstrated a synergistic antiviral effect of ribavirin and 6-MPTF against dengue virus infections in vitro (7). As a result of those studies, the chemotherapeutic efficacy of the combination of RTA and 6-MPTF against dengue virus infections in mice was evaluated. 6-MPTF alone or in combination with RTA was not effective in reducing dengue virus infections in mice, based on mean survival time and percent survival (group C, Table 1). In addition, when compared with RTA treatment alone, combined treatment with 6-MPTF caused a significant reversal (P < 0.001) of the antiviral efficacy of RTA. Recently, we have observed that 6-MPTF markedly reduces the proliferative responses of mononuclear leukocytes to mitogenic stimuli (W. C. Koff et al., unpublished data). The reversal of the therapeutic effect of RTA against dengue virus-infected mice by combined treatment with 6-MPTF might represent an immunosuppressive effect of 6-MPTF on certain populations of murine mononuclear leukocytes.

Rimantadine significantly inhibits dengue virus replication in vitro at concentrations well below cytotoxic levels (6). Using dosage levels comparable to those previously shown to be effective against influenza in mice and approximately 50% of the maximum tolerated dose (3), we conclusively demonstrated a lack of effect of rimantadine when administered i.p. against dengue virus infections in mice. This lack of efficacy might be due to an inability of rimantadine to cross the blood-brain barrier and reach the site(s) of viral infection.

Ribavirin is a nucleoside analog which exhibits antiviral efficacy against a substantial number of DNA and RNA viruses both in vitro and in vivo (9). However, previous reports have demonstrated that ribavirin is not effective against intracerebral virus infections of mice, regardless of the viral nucleic acid type, unless the drug is administered directly into the brain (1). This suggests that ribavirin or its active metabolites do not reach the brain in adequate concentrations. Our data showing a lack of effect of ribavirin when administered i.p. to dengue virus-infected mice concur with the hypothesis that the drug probably does not cross the blood-brain barrier.

RTA is a derivative of ribavirin with a greater index of lipid solubility. In previous studies, RTA has been found to be superior to ribavirin in the treatment of influenza-infected mice (12) and against arenavirus-induced hemorrhagic fever in rhesus monkeys (11). Because of its greater lipid solubility, RTA is more likely to pass through the blood-brain barrier and may act as a prodrug which is slowly hydrolyzed to ribavirin itself in vivo (2). Studies by Smee et al. (10) with Colorado tick fever virus infections supported this hypothesis of the propensity of RTA to cross the blood-brain barrier and suppress viral encephalitis disease. Our study demonstrates that RTA exhibits significant antiviral efficacy against dengue virus infections intracranially in mice when the drug is administered i.p. These encouraging results signal a need to further examine the efficacy of RTA against dengue viruses in subhuman primates, in which antiviral efficacy might be expected owing to the striking similarities in sites of virus replication between the dengue virus and arenavirus systems (4).

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