Therapeutic Effects of Small-Particle Aerosols of Ribavirin on Parainfluenza (Sendai) Virus Infections of Mice

E. W. LARSON,† E. L. STEPHEN, AND J. S. WALKER
United States Army Medical Research Institute of Infectious Diseases, Frederick, Maryland 21701

Received for publication 26 April 1976

Small-particle aerosol administration of ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) increased survival rates, extended the time to death, delayed lung pathology, and reduced pulmonary virus levels in Sendai virus-infected mice.

Infections with the parainfluenza viruses have been reported as a frequent cause of laryngotracheobronchitis in humans, with evidence that these infections account for at least one-third of the cases of croup in children (4). Parainfluenza viruses, types 1 and 2, have been identified most frequently in croup. Parainfluenza virus, type 1 (Sendai), is associated with naturally occurring respiratory infections of mice (5). The pathogenesis of these infections has been described (1, 6, 11). The disease process of Sendai virus infections in rodents closely parallels that seen with parainfluenza in children, and the rodent-Sendai system has been suggested as a suitable animal model for the study of the acute respiratory infections caused by parainfluenza in humans (3, 10).

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) has been shown to exhibit antiviral properties against parainfluenza virus types 1 and 3 in vitro (2, 7) and to be therapeutically effective in the treatment of type 1 (Sendai) infections in mice when the drug was administered intraperitoneally (7). In recent work, ribavirin was highly effective for the treatment of influenza type A infections in animals when administered therapeutically as small-particle aerosols (SPAs) (8, 9, 12). This report describes the therapeutic efficacy of ribavirin given as a SPA to mice infected with a lethal respiratory dose of Sendai virus.

Sendai virus, strain D/Sendai/52, was obtained from the American Type Culture Collection. Ten serial intranasal passages of the virus in mice yielded a virus isolate which, when propagated in the allantoic cavity of embryonated chicken eggs, was uniformly lethal for 5- to 7-week-old AKR/J mice after SPA challenge. Mice were exposed to aerosols of the mouse-adapted virus in each of three experiments. In each of the first two studies, one-half of the mice served as untreated, infected controls, whereas the remaining mice were treated continuously with ribavirin administered as SPA during the period from 24 through 72 h after virus challenge. The ribavirin was obtained from Nucleic Acid Research Institute of ICN Pharmaceuticals, Inc., Irvine, Calif. With only one continuous aerosol therapy apparatus, it was necessary to perform the third study to provide for an additional control. Again, one-half of the mice were infected and untreated, but the remaining mice were treated from 24 through 72 h with aerosolized, distilled water (the ribavirin diluent) in the continuous therapy apparatus.

The methods used for challenging mice with infectious virus and the procedures for continuous aerosol therapy have been described previously (8, 9). In the ribavirin studies, the concentration of drug in the spray suspension was adjusted to yield an effective, retained aerosol dose of 50 mg of ribavirin per kg (mouse weight) per day or 100 mg/kg in 48 h of treatment.

Three mice from each of the infected, untreated control and treatment groups were necropsied periodically through 10 days postchallenge in each experiment. The Sendai virus concentrations in lung, tracheobronchial and nasopharyngeal tissues were determined for each mouse and the extent of lung consolidation was scored. The methods used to quantify virus concentrations in mouse respiratory tissues have been described (8). Virus assays were performed in embryonated chicken eggs; tissue virus levels were measured in terms of median egg infectious doses/whole tissue. In one ribavirin experiment, 40 infected controls and 40 treated mice were challenged for observation of death patterns and time-to-death determinations. The same observations were
made on groups of 25 mice in the distilled water control study. All survivor mice were observed for a period of 21 days postinfection.

The mean Sendai virus levels in the respective mouse respiratory tissues from both ribavirin experiments are depicted in Fig. 1. The lung virus concentrations in the mice treated with ribavirin were significantly \((P < 0.05)\) lower than those in the infected controls for at least 120 h after treatment was discontinued (72 to 192 h). In contrast, virus levels in the tracheobronchial tissues of treated mice were significantly \((P < 0.05)\) below those in the infected controls only at 48 and 72 h. In this tissue, therefore, the effect of treatment did not persist, and the virus concentrations of treated mice approached control levels within 24 h after the termination of treatment. Somewhat lower virus concentrations in nasopharyngeal tissues of ribavirin treated mice were not significantly different from those in the infected, untreated control mice at all periods.

Table 1 summarizes the incidence of lung lesions among the treated and control mice as well as the survival and mean time-to-death properties among the mice which were caged separately for those purposes. Because lung lesions were not observed before 6 days and because in neither experiment did any of the untreated mice, which were designated for necropsy, survive beyond 8 days, statistical comparisons of the lung lesion data were not performed. The evidence suggested, however, that lung pathology developed later in the mice that received ribavirin than in the infected controls. Also, the results in Table 1 show the markedly better survival among the treated mice than among the untreated mice and show that the MTD of the mice that died after treatment was nearly 2 days greater than the MTD of those that died without treatment.

![Fig. 1. Effect of ribavirin continuous aerosol treatment (24 to 72 h) on Sendai virus levels (means and standard errors) in mouse respiratory tissues after lethal small-particle aerosol challenges with mouse-adapted virus. Symbols: ○, untreated; △, treated. *, Virus concentration with treatment significantly less than without treatment \((P \leq 0.05)\).](image)

Table 1. Lung lesion scores, survival, and mean time-to-death (MTD) properties of AKR mice after small-particle aerosol challenge with lethal doses of Sendai virus

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. of mice with lesions/total (mean score*)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lung lesions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 days</td>
<td>2/6(2.5)</td>
<td>ND</td>
</tr>
<tr>
<td>8 days</td>
<td>5/5(3.4)</td>
<td>3/6(2.7)</td>
</tr>
<tr>
<td>10 days</td>
<td>ND</td>
<td>6/6(3.7)</td>
</tr>
<tr>
<td><strong>Survivors (21 days), no./total (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0/40 (0)</td>
<td>25/39(64)</td>
</tr>
<tr>
<td><strong>Geometric MTD (days)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.1</td>
<td>9.8</td>
</tr>
</tbody>
</table>

* Mice showing lesions/total examined (mean lesion score of positives) based on a scale of 0 to 4 from negative to total consolidation.

* Treated continuously with ribavirin as small-particle aerosol from 24 through 72 h.

* No statistical test performed.

* No surviving mice.

* Chi-square test with Yates correction factor.

* Unpaired t test.
NOTES

were groups experiments were poses. by demonstrated that ribavirin, infections rus were the upper ment dai infections tant tissue studies. 12). ribavirin of Huffman, J. H., R. W. Appell, 1. L. B. Witkowski, in effect in L. B. Witkowski, acid and ribonucleic acid viruses. Antimicrob. Agents Chemother. 3:235-241.


Table 2. Whole tissue concentrations of Sendai virus in the respiratory tract of infected mice with and without distilled water aerosol treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hours postchallenge</th>
<th>Log EID$_{50}$/whole tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lung</td>
</tr>
<tr>
<td>Water$^a$ treated</td>
<td>48</td>
<td>4.44 (±0.24)$^c$</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>5.87 (±0.46)</td>
</tr>
<tr>
<td></td>
<td>168</td>
<td>4.80 (±0.17)</td>
</tr>
<tr>
<td>Untreated</td>
<td>48</td>
<td>3.85 (±0.33)</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>5.32 (±0.41)</td>
</tr>
<tr>
<td></td>
<td>168</td>
<td>4.17 (±0.29)</td>
</tr>
</tbody>
</table>

$^a$ EID$_{50}$, Median egg infectious dose.
$^b$ Treated continuously with distilled water aerosol from 24 through 72 h.
$^c$ ± Standard error (n = 3).

LITERATURE CITED


