Further Studies on Rabies Postexposure Prophylaxis in Mice: a Comparison of Vaccine with Interferon and Vaccine

(Accepted 30 August 1978)

SUMMARY

Mice were inoculated in the left hind footpad with street rabies virus, and 24 h later various types of rabies vaccine were administered intramuscularly in the right leg. The vaccines alone were ineffective in reducing mortality, but when an interferon inducer, polyriboinosinic acid-polyribocytidylic acid complexed to poly-L-lysine (polyICLC), was given along with the vaccines, a marked reduction resulted. The polyICLC-vaccine combination was effective even when it was injected 5 days after infection, suggesting that effective postexposure treatment in man might be successful when given at a comparable late time.

We have previously shown that in rabies virus infected mice the injection of a highly concentrated baby hamster kidney (BHK) cell rabies vaccine markedly reduced mortality (Baer & Cleary, 1972). Because of its ability to induce interferon, this vaccine could be diluted and still protect; equally immunogenic vaccines which were produced on human diploid cells, but which did not induce interferon, were ineffective (Baer & Yager, 1977). Also, the addition of local interferon or an interferon inducer to an ineffective vaccine resulted in marked protection (Baer et al. 1977). In the present study a potent interferon inducer and commercial rabies vaccines were administered to rabies-infected mice.

Female white Swiss mice, 4 weeks old, were divided into groups of 20 to 50 animals each for footpad challenge; weanling mice were used for intracerebral virus titration. We used viruses obtained from the salivary glands of rabid animals, namely a red fox (Vulpes fulva), kindly supplied by Ms Dora Woodall, Arizona Department of Health; a grey fox (see Baer & Yager, 1977); and an Arctic fox (Alopex lagopus), kindly supplied by Dr Don Ritter, Alaska Department of Health and Social Services.

The mice were injected in the left hind footpad with 0.03 ml of virus suspension diluted to produce approx. 50% mortality in the control animals. Human diploid cell vaccine (HDCV) with an antigenic value of 6·8 was kindly supplied by Dr R. Lang, Institut Mérieux, Lyon, France. The suckling mouse brain (SMB) vaccine (antigenic value 6·4) was prepared by the method of Fuenzalida & Palacios (1955) except that it was inactivated with β-propiolactone. Both vaccines were given as a single 0·1 ml intramuscular dose in the right hind leg.

Both polyICLC and mouse interferon were administered in 0.03 ml quantities in the left hind footpad. PolyICLC was prepared as previously described by Levy et al. (1975) and contained the equivalent of 2 mg poly(I). poly(C) in each ml. Mouse interferon, produced by stimulating C 243 cells with Newcastle disease virus (Oie et al. 1972a), was kindly supplied by Dr Sam Baron, NIH. Serum neutralizing titres were determined by the rapid fluorescent focus inhibition test (RFFIT; Smith et al. 1973) and the means calculated; six mice from each group were bled from the orbital sinus (Stone, 1954) on days 4, 8, 16 36 and 100. Average serum interferon titres were determined for 6 mice of each group 24 h after vaccination. Interferon was assayed by the method of Oie et al. (1972b). Mice were observed daily.
for 2 months and then three times a week for a year for signs of rabies; only those mice found positive by the fluorescent antibody technique (Dean, 1966) were recorded.

Mice were divided into three groups for the first study as follows: those given suckling mouse brain vaccine, those given suckling mouse brain and polyICLC and the control animals. In this study we wished to confirm that polyICLC and vaccine would protect more fully than vaccine alone. Treatment began 24 h after infection. The combination of polyICLC and SMB vaccine was again found to be much more effective than vaccine alone. The challenge killed 13 out of 42 controls; the mortality in the animals administered vaccine was 11 out of 42, while in those given polyICLC and SMB vaccine it was 3 out of 42. Similar effective treatment was seen in another study (Experiment A, Table 1) in which the addition of polyICLC or interferon to human diploid vaccine was compared to the use of vaccine alone. The reduction in the mortality rate was smaller when mice were inoculated with interferon plus HDCV.

In the next experiment the effect of delayed treatment with polyICLC + vaccine was compared to that of delayed amputation. Both polyICLC-SMB vaccine treatment and amputation were effective for 5 days after infection (Table 2). It should be noted that the amputation groups contained only 20 mice each.

In the last experiment the efficacy of polyICLC and SMB vaccine was tested against three different challenge virus doses (Experiment B, Table 1). Combined polyICLC-SMB vaccine treatment reduced the mortality from 58% to 32% after a challenge of 160000 MICLD₉₀ (median mouse intracerebral lethal dose); from 35.9% to 6.1% after a challenge of 16000 MICLD₉₀; and from 23% to 8.2% after a challenge of 1600 MICLD₉₀. SMB vaccine administered alone was not only ineffective but actually resulted in an increase in mortality – from 35.9% to 50% (Table 1). The mean interferon level in the treated mice was 10⁶⁹ reference units/ml 24 h after polyICLC administration and 3 out of 49 mice died in this group; the mean neutralizing antibody level was 10¹⁶ at 7 days. No interferon was noted in
Table 2. Mortality in mice treated with polyICLC and suckling mouse brain rabies vaccine or by amputation of the infected foot at different times after infection with Arctic fox challenge virus (10⁻⁵ dilution)

<table>
<thead>
<tr>
<th>Time after challenge (days)</th>
<th>Amputation</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0/20*</td>
<td>3/40</td>
</tr>
<tr>
<td>3</td>
<td>0/20</td>
<td>1/40</td>
</tr>
<tr>
<td>5</td>
<td>4/20</td>
<td>3/40</td>
</tr>
<tr>
<td>9</td>
<td>7/20</td>
<td>17/40</td>
</tr>
<tr>
<td>18</td>
<td>10/20</td>
<td>14/40</td>
</tr>
<tr>
<td>Controls</td>
<td>19/40</td>
<td></td>
</tr>
</tbody>
</table>

* Number dead/number tested.

the mice treated with SMB vaccine alone (mortality rate: 23/46), while the mean neutralizing antibody level was 10⁻¹⁸.

In previous studies in monkeys and mice, effective post-exposure protection was obtained only with rabies vaccine, prepared from a BHK cell substrate (Sikes et al. 1971; Baer & Cleary, 1972). Infected mice inoculated with BHK cell vaccine had detectable serum interferon 6 to 30 h later and there was a subsequent rise in rabies serum neutralizing antibody and a marked reduction in the mortality rate; this was noted even with vaccine diluted to the approximate potency of commercial rabies vaccines (Baer & Yager, 1977). In contrast, the rabies vaccines which only induced antibody were not effective.

Since a vaccine produced from heteroploid BHK cells cannot be used in man, we examined the effects of adding interferon or an inducer to commonly used commercial rabies vaccines. This combination had already been shown by Harmon & Janis (1975) to be effective in mice. PolyICLC is a nuclease-resistant complex able to withstand primate enzymes and it is an effective interferon inducer in non-human primates (Levy et al. 1975) and man (A. Levine, H. Levy and M. Lerner, personal communication). The polyICLC-vaccine combinations protected mice but vaccine alone was ineffective even though high levels of antibody were produced in both groups. These results confirm previous observations with duck embryo vaccine (Baer & Cleary, 1972) and human diploid vaccine (Baer & Yager, 1977) and show that the administration of vaccine alone (even one as potent as the suckling mouse brain product) does not reduce mortality in this model. The reduction in rabies deaths in groups of mice given polyICLC and vaccine was similar to that previously reported in mice and monkeys (Baer et al. 1977) and early and high levels of interferon followed by a rapid rise in neutralizing antibody were again detected. If such a combination of interferon inducer and vaccine can be safely given to humans, it should also prove to be highly effective.

In a previous mouse study we used a bobcat virus selected because of its ability to mimic the long incubation periods seen in man (Baer & Cleary, 1972) and showed that amputation of the inoculated foot markedly reduced mortality even when delayed for 18 days after infection. In the current study with a more rapidly invasive virus, amputation was not effective 9 days after exposure, but the dramatic reduction in the mortality rate among the mice treated on the 5th day after exposure (3/40 versus 19/40 in the control animals) indicates that the polyICLC-vaccine combination was still effective for as long as amputation was a life-saving procedure. It is thus probable that any effective treatment, including the polyICLC-vaccine combination, might still be effective up to a week following bites by a rabid animal.
Viral Zoonoses Branch
Virology Division, Bureau of Laboratories
Centre for Disease Control (Lawrenceville Facility)
Public Health Service
U.S. Department of Health, Education and Welfare
P.O. Box 363, Lawrenceville, Georgia 30246

* Present address: Sección de Rabia, Instituto Nacional de Investigaciones Veterinarias, Maracay, Venezuela.
† Present address: College of Veterinary Medicine, University of Georgia, Athens, Georgia, U.S.A.
‡ Present address: Laboratory of Viral Diseases, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md. 20014, U.S.A.
§ To whom reprint requests should be addressed, at Lawrenceville.

REFERENCES


(Received 4 May 1978)