The protective effect of the large-scale use of PHKC rabies vaccine in humans in China

Lin Fangtao

Reported are the results obtained with different immunization schedules of adjuvant or freeze-dried concentrated (FDC) primary hamster kidney cell (PHKC) rabies vaccine on volunteers. The FDC vaccine (potency, 4.5 IU), which was inoculated in six doses, on days 0, 3, 7, 14, 30 and 90, and the adjuvant vaccine (potency 2.5 IU), which was inoculated in five doses, on days 0 and 7 (double dose), 14, 30 and 90, induced earlier, higher, and more persistent neutralizing antibody titres than the adjuvant vaccine which was inoculated in five doses on days 0, 3, 7, 14 and 30. The persistence of the neutralizing antibody titres induced by three intradermal doses of vaccine administered on days 0 (4 sites), 7 (2 sites), and 28 (1 site) was lower than that induced by six intramuscular doses administered on days 0, 3, 7, 14, 30, and 90.

A cell-mediated immunity (CMI) was also induced in vaccinees who received the adjuvant vaccine. The protective effect of the adjuvant vaccine was better than that of the previously used Semple vaccine and has had a positive effect on the epidemiology of human rabies in China.

Introduction

Because of ethical, economic, and administrative problems, it is not easy to undertake mass immunization of animals against rabies in many developing countries, including China. Postexposure treatment is, therefore, still a significant means of controlling human rabies, and the economical adjuvant vaccine is well-suited for use among humans in this setting.

Both the neutralizing antibody titre and the protective effect of primary hamster kidney cell (PHKC) rabies vaccine (with or without adjuvant) in humans have been determined (1–4), and several years of field trials with both pre- and postexposure inoculation in China indicate that the vaccine is safe and effective. In these trials, 228 volunteers received preexposure inoculation; and 301 patients were given postexposure treatment, most of whom had been bitten by animals with laboratory-proven rabies, while the remainder had been bitten by animals suspected to have rabies and that had escaped. Of the patients, 58 had been bitten by a rabid wolf or feral dog (4, 5).

Here we report the neutralizing antibody titres and cell-mediated immunity (CMI) responses in humans vaccinated with different immunization schedules of rabies vaccine. Data are also provided on the protective effect of the adjuvant vaccine used in large-scale postexposure treatment and its effect on the epidemiology of human rabies in China.

Materials and methods

Vaccine

Two types of PHKC vaccine (adjuvant and freeze-dried concentrated (FDC)), which were developed from an adapted Beijing strain of a fixed rabies virus grown in primary hamster kidney cells, were employed in the study. The potency of the adjuvant vaccine was determined using the Habel test (index, 100,000) or the NIH test (2.5 IU), and that of the FDC vaccine using the NIH test (2.5 IU).

A dose of purified horse immune serum (neutralizing antibody titre = 1:2500; WHO Reference Animal Serum, 1:2188) equivalent to 40 IU/kg body weight (0.5 ml/kg body weight) was injected simultaneously with the vaccine.

Neutralizing antibody titre

Samples of sera from vaccinees were taken on days 0, 7, 21, 45 (or 65), 105, and 365, and the neutralizing antibody titre was determined using the method described by Atanasiu (6). The Beijing strain of fixed virus (289–290 passages) or the CVS strain was used as the challenge virus (32–320 LD50).

Cell-mediated immunity

Adjuvant vaccine (Lot 83–430; Habel index, 363 160) induced cell-mediated immunity, as indicated by the results of the E-rosette forming and lymphocyte transformation tests (7, 8).

Diagnosis

Human rabies was diagnosed clinically by physicians in local basic health units located in rural areas and in local country or town hospitals.
Vaccinees and observation design (pre-exposure immunization)

Vaccinees. Physicians selected volunteers of both sexes aged 16 years or more who were in good health and who had no previous history of rabies vaccination. The subjects were immunized using several vaccination schedules, doses, and inoculation routes. Every vaccinee was allocated randomly to one of the immunization regimens.

Postexposure treatment. Persons bitten by suspected rabid animals were treated by local physicians in the provinces of Jiangsu, Anhui, Jiangxi, and Hunan.

For mildly exposed persons, vaccine was given on days 0, 3, 7, 14 and 30, and for severely exposed persons, on days 0, 3, 7, 14 and 30, with one or more boosters on day 90 or on days 40, 50, and 120.

Results

Neutralizing antibody responses with PHKC vaccine

The neutralizing antibody responses for the recipients of the five-dose or six-dose immunization schedules are shown in Table 1. The adjuvant vaccine (potency 2.5 IU) or FDC vaccine (potency 4.5 IU) induced a good antibody response on day 21 and high level titres on day 45. The responses were more rapid and longer lasting when the adjuvant vaccine was administered on days 0 (double dose) and 7 (double dose), and the FDC vaccine on days 0, 3, 7, 14, 30, and 90.

Neutralizing antibody responses with PHKC or PCEC vaccine

Two different vaccines—PHKC vaccine (potency, 4.9 IU or 7.3 IU) and purified chick-embryo cell (PCEC) vaccine (potency, 7.5 IU)—were administered intramuscularly or intradermally. As shown in Table 2, the neutralizing antibody titres of the PHKC vaccine (potency, 7.3 IU) and PCEC vaccine (potency, 7.5 IU) developed more rapidly and were higher than those of the PHKC vaccine (potency, 4.9 IU).

Irrespective of whether PHKC or PCEC vaccine was used, the titres on days 105 and 365 were higher following six intramuscular doses than following three intradermal doses.

Cell-mediated immunity response to the adjuvant PHKC vaccine

The cell-mediated immunity response to the adjuvant PHKC vaccine on days 7, 21, 45, 120, and 365 after six doses is shown in Fig. 1.

Postexposure protection provided by the adjuvant PHKC vaccine

The postexposure treatment schedule for the PHKC vaccine was used on days 0, 3, 7, 14, and 30 for mildly exposed patients, with a booster on day 90, or

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of vaccinees</th>
<th>Vaccine/atri</th>
<th>Potency (IU)</th>
<th>Immunization schedule (days)</th>
<th>Reciprocal geometric mean titre on days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>17</td>
<td>287*</td>
<td>2.5</td>
<td>0-3, 7, 14, 30</td>
<td>&lt;5</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>287</td>
<td>2.5</td>
<td>0-3, 7, 14, 30, 90</td>
<td>&lt;5</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>287</td>
<td>2.5</td>
<td>0 x 2, 7 x 2* 14, 30, 90</td>
<td>&lt;5</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>85-5*</td>
<td>4.5</td>
<td>0-3, 7, 14, 30, 90</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

* Adjuvant vaccine
a Figures in parentheses are the percentage who seroconverted (reciprocal titre >5-10).
* Figures in italics are the range
* Double dose
* Freeze-dried concentrated vaccine.
booster on days 40, 50, and 120 for those who were severely exposed. Over the period 1979–84, we studied retrospectively the protection provided by the PHKC vaccine and compared it with that of the Semple vaccine, which had been used to vaccinate 47,903 persons exposed to rabid or suspectedly rabid animals in the following endemic areas: Jiangshu and Anhui provinces (Table 3) and in Guangxi autonomous region and Hunan province (Table 4). The data confirmed that the protection provided by adjuvant PHKC vaccine was significantly greater than that induced by the Semple vaccine.

The protective effect of the adjuvant vaccine was also studied retrospectively in Hubei province. In 1986–87, 26 cases of human rabies occurred and 389 persons were exposed to rabid or suspectedly rabid dogs; each dog bit 10–30 persons over a 2-day period. Among these individuals, 10 deaths from rabies occurred among 110 patients who had been bitten by one of 9 rabid dogs. Table 5 shows that 9 out of the 10 rabies victims who died had been severely bitten; however, a combination of antiserum and vaccine was given to only two of these individuals, and no one received proper wound treatment.

A mildly exposed child, who received five doses of vaccine, died 176 days after being bitten. This victim could possibly have survived had a booster dose been given.

**Epidemiological effect of postexposure treatment with PHKC vaccine**

Data on human postexposure treatment and rabies deaths were obtained for Jiangxi province (Fig. 2) and also for China as a whole (Fig. 3).

From October 1980 to October 1987, approximately 10 million persons in China received PHKC vaccine—mainly the adjuvant type—for postexposure treatment of rabies. The large-scale use of the
Lin Fangtao

Table 3. Protectivity provided by postexposure treatment with adjuvant PHKC vaccine to persons bitten by suspected rabid animals, compared with that provided by the Sample vaccine, in Jiangsu and Anhui provinces

<table>
<thead>
<tr>
<th>Province</th>
<th>Date</th>
<th>No. of persons</th>
<th>No. with rabies</th>
<th>PHKC vaccine</th>
<th>Sample vaccine</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No. of persons</td>
<td>No. with rabies</td>
<td>No. of persons</td>
</tr>
<tr>
<td>Jiangsu</td>
<td>1979–82</td>
<td>6850</td>
<td>9 (0.13)</td>
<td>4486</td>
<td>18 (0.4)</td>
<td>808</td>
</tr>
<tr>
<td>Anhui</td>
<td>1979–81</td>
<td>3138</td>
<td>6 (0.19)</td>
<td>1525</td>
<td>9 (0.59)</td>
<td>2269</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>10,080</td>
<td>15 (0.15)</td>
<td>6011</td>
<td>27 (0.45)</td>
<td>3077</td>
</tr>
</tbody>
</table>

* Student's t-test = 3.6, P < 0.001
b Figures in parentheses are the percentage of treatment failures

Table 4: Protectivity provided by postexposure treatment with adjuvant PHKC vaccine to persons bitten by suspected rabid animals in Guangxi autonomous region and Hunan province

<table>
<thead>
<tr>
<th>Location</th>
<th>Date</th>
<th>No. of persons</th>
<th>No. with rabies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanxi and Hunan</td>
<td>1981</td>
<td>10,000</td>
<td>2 (0.02)</td>
</tr>
<tr>
<td></td>
<td>1982</td>
<td>7165</td>
<td>8 (0.11)</td>
</tr>
<tr>
<td></td>
<td>1983–84</td>
<td>20,668</td>
<td>11 (0.048)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>37,823</td>
<td>21 (0.055)</td>
</tr>
</tbody>
</table>

* Figures in parentheses are the percentage of treatment failures

vaccine appears to have influenced the epidemiology of the disease in Jiangxi province and, to some extent, also in the entire country.

Discussion

Despite the availability of several types of safe, potent cell-culture rabies vaccines (9–16), rabies vaccine produced in the brain tissue of adult animals is still widely used in some developing countries.

There have been many appeals that a solution be sought to this problem. For example, at a WHO Consultation in Essen, FRG, it was stated that "the problem that is giving us cause for concern is how to help the developing world in the production of safer and more potent vaccine than they are using at present" (17). Subsequently, Bögel recommended

Table 5: Details of the 10 deaths from rabies in Hubel province

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>Class of exposurea</th>
<th>No. of days after biting</th>
<th>Wound</th>
<th>Dose of vaccine</th>
<th>Dose of serum</th>
<th>No. of days' incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. M</td>
<td>11</td>
<td>II</td>
<td>1</td>
<td>Cleansed</td>
<td>× 5</td>
<td>NT</td>
<td>175</td>
</tr>
<tr>
<td>2. M</td>
<td>17</td>
<td>III</td>
<td>NTa</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>23</td>
</tr>
<tr>
<td>3. F</td>
<td>14</td>
<td>III</td>
<td>20</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>28</td>
</tr>
<tr>
<td>4. M</td>
<td>9</td>
<td>III</td>
<td>20</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>29</td>
</tr>
<tr>
<td>5. F</td>
<td>16</td>
<td>III</td>
<td>30</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>43</td>
</tr>
<tr>
<td>6. M</td>
<td>7</td>
<td>III</td>
<td>1</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>39</td>
</tr>
<tr>
<td>7. M</td>
<td>37</td>
<td>III</td>
<td>1</td>
<td>NT</td>
<td>× 5</td>
<td>NT</td>
<td>41</td>
</tr>
<tr>
<td>8. M</td>
<td>30</td>
<td>III</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>93</td>
</tr>
<tr>
<td>9. F</td>
<td>47</td>
<td>III</td>
<td>1</td>
<td>Cleansed and stitched</td>
<td>× 5</td>
<td>2000 IU</td>
<td>30</td>
</tr>
<tr>
<td>10. F</td>
<td>25</td>
<td>III</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>70</td>
</tr>
</tbody>
</table>


b NT = not treated
neutralizing antibody response. Although the adjuvant added to the vaccine can delay the response, this difficulty can be overcome by increasing the dosage on day 0 or by using high-potency adjuvant vaccine, or antiserum and vaccine in combination, for all postexposed patients, irrespective of whether they have been mildly or severely bitten.

The adjuvant rabies vaccine that was licensed in China in 1980 has completely replaced the Semple vaccine; more than 50 million doses have been produced, and these have mainly been used for postexposure treatment. In China, the protective effect of the adjuvant vaccine has not only been confirmed in two incidents involving rabid wolves but also has been used for millions of postexposure treatments.

The potent, stable and well-tolerated adjuvant, concentrated PHKC vaccine and FDC vaccine for alternative use were also licensed in China in 1980, and approximately 1 million doses have been produced and used for postexposure treatment. We are now planning to purify the adjuvant concentrated vaccine and the FDC vaccine.

Résumé
Effet protecteur de l'utilisation à grande échelle du vaccin antirabique PHKC (obtenu en cultures primaires de cellules rénales de hamster) chez l'homme en Chine

L'article décrit les résultats obtenus avec divers calendriers d'administration à des volontaires de vaccin antirabique obtenu en cultures primaires de cellules rénales de hamster (PHKC) soit adjuvé, soit concentré et lyophilisé (FDC). Le vaccin concentré lyophilisé (activité 4,5 UI) inoculé en six doses les jours 0, 3, 7, 14, 30 et 90, et le vaccin adjuvé (activité 2, 5 UI) inoculé en cinq doses les jours 0 et 7 (double dose), 14, 30 et 90, induisaient des titres d'anticorps neutralisants plus rapidement obtenus, plus élevés et plus durables que le vaccin adjuvé inoculé en cinq doses les jours 0, 3, 7, 14 et 30. La persistance des titres d'anticorps neutralisants induits par trois doses intradermiques de vaccin administrées les jours 0 (4 sites), 7 (2 sites) et 28 (un site) était plus faible que dans le cas de six doses intramusculaires administrées les jours 0, 3, 7, 14, 30 et 90.

Une immunité à médiation cellulaire (CMH) a également été induite chez les sujets ayant reçu le vaccin adjuvé. L'effet protecteur de ce dernier était meilleur que celui du vaccin Semple utilisé auparavant et a eu un effet positif sur l'épidémiologie de la rage humaine en Chine.
References


