Evaluation of two intradermal vaccination regimens using purified chick embryo cell vaccine for post-exposure prophylaxis of rabies

S. N. MADHUSUDANA, N. PREM ANAND, RANJANI SHAMSUNDAR

ABSTRACT

Background. Post-exposure prophylaxis for rabies with cell culture vaccines by the conventional intramuscular regimen is very expensive. The World Health Organization has advocated two cost-effective intradermal regimens with cell culture vaccines for use in developing countries. We evaluated these two regimens—the 2-site and the 8-site regimens—in terms of immunogenicity, safety and tolerance in people with category I exposure to rabies.

Method. Eighty-two subjects who had mild category I exposure to rabies were immunized using a purified chick embryo cell vaccine. The first regimen given to 43 subjects, consisted of intradermal administration of 0.2 ml of vaccine at 2 sites on days 0, 3 and 7 and at one site on days 28 and 90. The second regimen, given to 39 subjects, consisted of intradermal administration of 0.1 ml of vaccine at 8 sites on day 0, at 4 sites on day 7 and at one site on days 28 and 90. The mouse neutralization test was used to estimate titres of rabies neutralizing antibody in these subjects on different days after vaccination. The subjects were followed up for 1 year.

Results. Both regimens produced adequate neutralizing antibody titres from day 14 onwards, though the second regimen produced a more rapid antibody response and significantly higher titres (p < 0.001) on all days tested. There were minimal side-effects and both regimens were well tolerated.

Conclusion: Both the 2-site and 8-site intradermal regimens with purified chick embryo cell vaccine produce adequate levels of neutralizing antibodies but the 8-site regimen appears to be more immunogenic. The feasibility of using these cost-effective regimens in routine practice needs to be further evaluated under the field conditions prevalent in India.

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INTRODUCTION

According to the latest World Health Organization (WHO) report, worldwide about 60 000 people die of rabies encephalitis every year, mostly in the developing countries of Asia and Africa. Of these, 30 000 (50%) deaths are from India. Several factors are responsible for the continued prevalence and endemicity of the disease in India. The most important is the continuing stray dog menace and the use of the highly reactogenic Semple vaccine which has poor patient compliance due to the fear of taking multiple painful injections in the abdomen. Though cell culture vaccines (CCVs) such as the human diploid cell (HDC) vaccine, purified chick embryo cell (PCEC) vaccine and purified Vero cell rabies (PVR) vaccine are popular among the more affluent and educated, a majority of people attending government hospitals and dispensaries are still administered the sheep brain-derived Semple vaccine. This vaccine sometimes causes severe neurological complications in addition to frequent local reactions.

The WHO has strongly recommended discontinuーション of the use of nerve tissue-derived vaccines and their complete replacement with safe and potent CCVs. As the conventional intramuscular dosage schedule with CCVs is expensive for developing countries, WHO has recommended the use of economic and cost-effective intradermal (i.d.) regimens in these countries. One i.d. regimen which has undergone extensive clinical trials and is now routinely used in Thailand is the 2—2—2—0—0—1—1 regimen, which has already been administered to more than 70 000 people without any reported failures. In India, rabies is a major public health problem and there is a need to study the efficacy of the economical i.d. regimens. The antibody response to this regimen using the PCEC vaccine was earlier reported from this laboratory. The WHO also advocates another multi-site regimen to be used with the PCEC, HDC and purified duck embryo (PDE) vaccines, whose recommended intramuscular (i.m.) dose is 1 ml. In the present study, this regimen was evaluated in comparison to the two-site regimen.

SUBJECTS AND METHODS

Eighty-two medical and paramedical staff working in the neurological services of the National Institute of Mental Health and Neurosciences, Bangalore who had nursed or treated 5 patients with suspected Guillain-Barré syndrome and were later diagnosed to have paralytic rabies (based on autopsied brain samples subjected to direct immunofluorescence and virus isolation procedures) were identified. It was decided to immunize these subjects with the PCEC vaccine as they had had a potential though low-risk exposure to rabies. The study sample had 42 adult men and 40 adult women.

Vaccine and regimen

The PCEC vaccine used had a potency of 7.8 IU per ml by the National Institutes of Health, USA tests (personal communication from the manufacturer). The vaccine was reconstituted with 1 ml of diluting fluid provided by the manufacturer, and used on the same day to vaccinate the subjects. Forty-three (20 men and 23 women) subjects received the first regimen in which 0.2 ml of vaccine was given i.d. at 2 sites (over the deltoid region) on days 0, 3 and 7 and at one site on days 28 and 90. Thirty-nine subjects (22 men and 17 women) received the second regimen in which 0.1 ml of vaccine was administered at 8 sites (deltoid 2, infrascapular 2, abdomen 2 and anterior part of the legs 2) on day 0, at 4 sites on day 7 and at 1 site on days 28 and 90. Subjects were randomly assigned to receive either of the 2 regimens, and were informed about the use and efficacy of the i.d. schedule recommended by WHO. Their consent was taken both for vaccination and blood testing. Blood was collected on days 0 (before vaccination), 7, 14 and 90, at 6 months and after 1 year. The serum was separated and frozen at −75 °C pending assay for antibody titres.

Estimation of neutralizing antibody titres

This was done by performing standard mouse neutralization tests...
as advocated by WHO. The challenge virus standard strain (CVS) was procured from the Central Research Institute, Kasauli and was subsequently passaged twice in mice to prepare in-house batches of CVS which had a titre of 10 MICLD 50 (mouse intracerebral lethal dose) per 0.03 ml. A dose of 100 LD 50 was used for the test. The titres obtained in terms of dilutions of serum showing 50% survival were expressed in IU per ml and compared to an in-house preparation of reference rabies immunoglobulin (RIG) with a potency of 60 IU per ml when calibrated against the first WHO reference preparation of RIG with a potency of 59 IU per ml.

Statistical analysis

The difference in the geometric mean titres obtained on each day of sampling between the groups was analysed using a two-tailed Student t-test and a p value was obtained for each day of sampling.

RESULTS

None of the subjects had rabies neutralizing antibody titres on day 0 before vaccination. By day 14, all subjects administered both the regimens developed titres much higher than the minimum level of 0.5 IU/ml indicating seroconversion. However, the titres obtained with the second regimen were significantly higher (p<0.05 to p<0.001) in all those tested (Table I). Also, with the second regimen there was a more rapid antibody response, with titres much higher than the minimum level indicating seroconversion. However, the titres obtained in terms of dilutions of serum above 0.5 IU/ml by day 7. With both the regimens, adequate titres were present even at the end of the one-year follow up period. Both the regimens were well tolerated, and side-effects such as itching, pain and induration at the injection site were minimal (Table II).

DISCUSSION

We evaluated the neutralizing antibody response to two cost-effective i.d. regimens with the PCEC vaccine, keeping in view the need to have such a regimen for post-exposure treatment of rabies.

<table>
<thead>
<tr>
<th>Vaccine regimen Neutralizing antibody titres (IU/ml)*</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 90</th>
<th>Day 180</th>
<th>Day 365</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-site (n=43)</td>
<td>0.22</td>
<td>6.8</td>
<td>5.8</td>
<td>2.3</td>
<td>0.9</td>
</tr>
<tr>
<td>8-site (n=39)</td>
<td>0.85</td>
<td>10.2</td>
<td>8.5</td>
<td>4.6</td>
<td>2.6</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Values are geometric mean titres of the groups compared by two tailed t-test

TABLE II. Side-effects observed in subjects vaccinated with two different intradermal regimens using the purified chick embryo cell vaccine

<table>
<thead>
<tr>
<th>Side-effect</th>
<th>2-site regimen (n=43)</th>
<th>8-site regimen (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>4 (9.3)</td>
<td>3 (7.6)</td>
</tr>
<tr>
<td>Induration</td>
<td>5 (11.1)</td>
<td>4 (10.2)</td>
</tr>
<tr>
<td>Itching</td>
<td>4 (9.3)</td>
<td>3 (7.6)</td>
</tr>
<tr>
<td>Erythema</td>
<td>4 (9.3)</td>
<td>3 (7.6)</td>
</tr>
<tr>
<td>Fever</td>
<td>1 (2.4)</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Headache</td>
<td>1 (2.4)</td>
<td>1 (2.3)</td>
</tr>
</tbody>
</table>

Figures in parentheses are percentages

The subjects included in our study had nursed patients with rabies and were apprehensive about the possible risk of contracting the disease. Human-to-human transmission of rabies is considered to be only a theoretical possibility,11 barring a recent report from Africa of possible man-to-man transmission.12 As the risk involved in these subjects was low, it was decided to administer post-exposure treatment with i.d. regimens. All the subjects developed adequate antibody titres which persisted till the end of the one-year follow up period.

The presence of adequate levels of neutralizing antibodies seems to correlate best with protection against rabies.13 The minimum level indicating seroconversion is thought to be 0.5 IU/ml.7 The speed of response is critical since many treatment failures occur either due to delay in starting treatment or failure to administer passive immunization with RIG.14,15 The multisite administration of CCVs accelerates and increases the antibody response. The 8-site regimen also has a good margin of safety. Even if two i.d. doses are given subcutaneously due to faulty administration, a good immune response can be obtained.16 We did not administer RIG as the risk involved was minimal. However, as reported earlier, simultaneous administration of RIG does not significantly affect the antibody response to both the 2-site and 8-site regimens.4,16

The batch of vaccine used by us had a potency of 7.8 IU/ml, which is much more than the minimum potency of 2.5 IU/ml prescribed by WHO. It is essential to evaluate these low-dose regimens with vaccines having marginal potency values before one can safely recommend them for use. Khawplod et al.17 reported that with the use of the PDE vaccine, a minimum antigen content of 5 IU/ml may be necessary for effective immune response. However, in our earlier study of the 2-site i.d. regimen with PCEC vaccine,7 we obtained more than adequate antibody titres by using half the amount of vaccine when we dissolved it in 1 ml diluent and administered only 0.1 ml at each site with a 2-2-2-0-1 regimen. In Thailand, this regimen is popular with PVR vaccine. More studies may be required to determine the minimum potency that is advisable with the use of i.d. regimens. Another important aspect that needs to be studied is the effect of concomitant administration of chloroquine and other immunosuppressive drugs, malnutrition and altered immune conditions. Antibody response was not adequate in subjects who took pre-exposure prophylaxis by the i.d. route with 0.1 ml of vaccine and concomitantly took chloroquine for prophylaxis of malaria.18,19

We demonstrated that an adequate immune response can be obtained with the PCEC vaccine using both the 2-site and 8-site regimens, with nearly 60% saving on the quantity of vaccine used. However, these regimens are more economical and useful in centres where many cases of animal bites are seen on a single day so that the reconstituted vaccine can be evenly distributed among the patients with no leftover, as storage of the reconstituted vaccine for more than 6–8 hours is not recommended.

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REFERENCES


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