Economical multi-site intradermal regimen with purified chick embryo cell vaccine (Rabipur) prevents rabies in people bitten by confirmed rabid animals

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**Objective:** To determine the efficacy of a cost-effective multi-site intradermal regimen with purified chick embryo cell vaccine (PCECV, Rabipur) in preventing rabies in people bitten by confirmed rabid dogs.

**Methods:** Thirty-two people of different age groups who were severely bitten by confirmed rabid dogs were immunized with PCECV using the WHO recommended multi-site intradermal regimen of 0.1 mL of vaccine at eight sites on day 0, at four sites on day 7, and at one site each on days 28 and 90. In addition, passive immunization with human or equine rabies immunoglobulin was administered to 22 of these people before administering vaccine. They were followed for up to 3 years with periodic estimation of neutralizing antibody levels in their serum by mouse neutralization test (MNT).

**Results:** There was an excellent immune response with more than protective titers (>0.5 IU/mL) on all days tested up to the end of the 3-year observation period. More significantly, protective titers were seen in all subjects by day 7. Only minimal side effects were observed. All the patients were doing well at the end of the 3-year observation period, which is generally considered to be the maximum incubation period for rabies in humans.

**Conclusions:** It can be concluded that this multi-site regimen with or without passive immunization has prevented the development of rabies encephalitis in these people bitten by confirmed rabid dogs. This should encourage more such studies, so that this cost-effective economical regimen with safe and potent cell culture vaccines can replace highly reactogenic neural tissue-derived Semple vaccine in developing countries such as India.

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**INTRODUCTION**

Rabies continues to be a serious public health problem in many Asian, African and South American countries. The worldwide incidence of human rabies is estimated to be around 60,000, with 30,000 human deaths reported from India alone. In India, the continuing stray dog menace and the non-availability of modern cell culture vaccines (CCVs) in government-run hospitals are the two main factors responsible for the situation. About 0.5 million people undergo post-exposure vaccination; more than two-thirds receive sheep brain derived Semple vaccine, and less than one-third receive CCVs. Rabies post-exposure treatment with CCVs such as human diploid cell vaccine (HDCV), purified chick embryo cell vaccine (PCECV) or purified Vero cell rabies vaccine (PVRV) is very expensive if a regular intramuscular dosage schedule is followed. Because of this, the majority of people bitten by rabid dogs prefer to take multiple doses of nerve tissue-derived Semple vaccine, which is administered free of charge in government hospitals and dispensaries. Apart from causing local reactions like induration and abscess formation in many vaccinees, this vaccine can also produce severe neurologic complications in as many as 1 in 5000 cases. Another drawback of this vaccine is the poor patient compliance leading to inadequate immunization and vaccine failures. It may take several years to introduce safe and potent CCVs in government-run hospitals. Many of the educated class, who are aware of the dangers of Semple vaccine, opt for modern CCVs despite the fact the whole treatment procedure may be very costly. The total cost of treatment with these vaccines in many cases may be more than the monthly earnings of the majority of people bitten by street dogs.

In developing countries such as India, as modern CCVs are not administered free of cost, there is scope for the introduction of cost effective intradermal (ID) regimens, which are now gaining increasing popularity in countries like Thailand. Presently, the WHO recommends a two-site regimen with PVRV and an eight-site regimen with PCECV and HDCV. By the use of these economical regimens, up to 65% of vaccine can be saved in comparison to conventional intramuscular regimens. Several studies, from both India and elsewhere, have

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consistently demonstrated good immunogenicity of two-site and eight-site ID regimens.5,7-9 The earlier studies reported from India have not addressed the efficacy of ID regimens in preventing rabies in patients truly exposed to rabies, though these studies have confirmed good humoral responses to these regimens.9,10 In this paper, for the first time from India we report the efficacy of a multi-site ID regimen in preventing rabies in patients who were severely bitten by confirmed rabid animals.

MATERIALS AND METHODS

Patients

Thirty-two patients of various ages and of both sexes who were bitten by dogs attended the department of Neurovirology, National Institute of Mental Health and Neuroscience (NIMHANS), Bangalore (India) for expert post-exposure advice during the period from January 1994 to December 1995. Among these, 14 were adult males, eight were adult females, and 10 were children aged between 7 and 13 years. The source of exposure comprised 12 dogs who had inflicted multiple bite injuries. Among these, nine were stray dogs and three were pet dogs but unimmunized. Three dogs had bitten only one patient each, but the remaining nine had bitten two to four persons at the same time. Subsequently, these animals were killed and their brains were brought to this laboratory for confirmation of rabies. Eighteen patients had multiple transdermal bites, three children had severe lacerated wounds on their faces, five had lacerated wounds on their forearms, and the remaining had one or two transdermal bites on forearms or legs. Classification as per WHO11 revealed that all of them had category III exposure. All of these patients were otherwise healthy and not on any medication, including immunosuppressive drugs.

Diagnosis of rabies in dogs

Rabies was confirmed in all the 12 dogs by direct immunofluorescence examination of brain, as per the standard procedure.12 The conjugate used was obtained from the Central Research Institute, Kasauli, (India). The brains of all the dogs tested had florid distribution of viral antigen in all the areas of brain tested. Subsequently, the results were also confirmed by mouse inoculation test.13 The incubation period of these strains in mice varied from as short as 7 days to a maximum of 12 days.

Management of patients

Adequate local wound toilet and a dose of tetanus toxoid had been administered to 24 of these patients immediately after the bite by the local medical practitioner. The remaining patients did not receive immediate local treatment. All of these had received a dose of tetanus toxoid before presenting to us. The wound was not sutured in any patient, although in six cases severe lacerated injury was present. Passive immunization was administered to 22 patients within 3 days after the bite. Among these, 10 received equine rabies immunoglobulin (ERIG, Pasteur-Merieux Connaught, Leon, France) at a dose of 40 IU/kg body weight, and 12 patients received human rabies immunoglobulin (HRIG, Behring, Marburg, Germany) at a dose of 20 IU/kg body weight. The remaining 10 patients did not receive serum therapy, as they were very poor and could not afford to purchase either ERIG or HRIG from the market. Before administering ERIG to these patients, serum sensitivity was tested as recommended by the manufacturer, following the usual precautions, but none of our patients was found to be sensitive. As far as possible, the total calculated dose of hyperimmune serum was infiltrated in and around the wounds, and the remainder was given deep intramuscularly. All of these patients (parents in the case of children) were verbally told about the known efficacy and usefulness of the multi-site ID regimen, and written consent was obtained for both the ID regimen and testing of blood samples. All of these patients were then given 0.1 mL of PCECV (Rabilpur, Hoechst India Ltd, Mumbai, India) batch numbers 255 and 265, with potencies of 7.6 IU/mL and 7.8 IU/mL respectively, by NIH test (personal communication from Hoechst India Ltd) intradermally at eight sites on day 0, at four sites on day 7, and at one site on days 28 and 90. In most cases, we were able to adhere to the sites recommended by the WHO, i.e. one each over deltoids, suprascapular regions, lower quadrant of abdomen, and lateral aspects of legs or thighs, but in some we had to alter the sites, depending on the distribution of wounds around which hyperimmune serum was infiltrated. Antibiotics were prescribed to 12 of these patients, as they had a lot of soft tissue damage.

Estimation of neutralizing antibody titers

The patient’s sera were titrated for rabies-neutralizing antibodies on days 0, 7, 28 and 90, and at 1-, 2- and 3-year intervals by performing the standard mouse neutralization test as advocated by the WHO.14 The challenge virus standard (CVS) was obtained from the Central Research Institute, Kasauli. The titers were expressed in IU/mL in comparison to a local reference preparation of rabies immunoglobulin (RIG) previously calibrated against the first WHO international reference RIG with 59 IU/mL.

Statistical analysis

The differences in the antibody titers seen in the groups with and without passive immunization were analyzed by applying Student’s t-test.

Follow-up of patients

Since all of these patients were from Bangalore, they were regularly followed, with instructions to report to us
for collection of blood at regular intervals until the end of the third year post-vaccination.

RESULTS

Neutralizing antibody titers

None of our patients had detectable levels of antibodies on day 0 before administration of vaccine. The titers of neutralizing antibodies from day 7 to the end of the 3-year follow-up period are shown in Table 1. It can be seen that more than protective titers (>0.5 IU/mL) were present in all these patients from day 7, with more than adequate titers on days 28 and 90. Protective titers persisted until the end of the 3-year observation period. Furthermore, there was no significant difference in the titers observed between the groups with or without passive immunization (Table 2).

Vaccine tolerance and side effects

All of the patients, including 10 children, tolerated multiple ID inoculations. The side effects observed were mostly insignificant, and comprised itching or pain at the inoculation sites, occasional induration and erythema, mild fever, and cervical adenopathy in one case. These effects disappeared spontaneously in the majority of cases. Only two patients were prescribed mild analgesics or antipyretics.

Follow-up of cases

The patients were followed for 3 years. Most of the patients reported on their own on specified dates. Some had to be repeatedly contacted via telephone or personally. All of them were alive and doing well at the end of the observation period.

DISCUSSION

The development of rabies in non-immunized people after a genuine exposure is reported to vary from 15% to 60%, depending on several factors, such as quantum of virus inoculated, nature of wound, proximity to brain and spinal cord, body surface area of the victim, and as yet unknown reasons. All of the patients in this study had severe bite wounds, often multiple, and in four cases over the face; in all cases, the diagnosis of rabies in the biting animal was confirmed. Therefore, we expect that, without adequate immunization, 60% of these, i.e. 16 people, might have developed rabies. The incubation period of rabies is also variable, depending on severity and site of exposure, quantum of virus inoculated, and probably as yet unidentified factors. However, it is well established that the incubation period in severe class III exposures may range from as little as a few days to a maximum of 3–6 months. Since all of these patients had severe class III exposure, we would expect incubation period in these cases to be a few days to a few weeks, and in any case not more than 6 months. The patients in this study were followed up for 3 years, which is considered to be the longest incubation period. At the end of this period, all of our patients were alive and doing well. They still had protective levels of antibodies at that time. Therefore, we believe that timely administration of vaccine by the ID route and passive immunization as per the recommended schedule has prevented the development of rabies in these patients.

Table 1. Rabies neutralizing antibody titers at different time intervals after ID immunization with PCECV

<table>
<thead>
<tr>
<th>Patient group</th>
<th>On days</th>
<th>At the end of</th>
<th>1st year</th>
<th>2nd year</th>
<th>3rd year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
<td>28</td>
<td>90</td>
<td>1st year</td>
<td>2nd year</td>
</tr>
<tr>
<td>Adult Males</td>
<td>1.8</td>
<td>8.5</td>
<td>7.5</td>
<td>4.2</td>
<td>1.8</td>
</tr>
<tr>
<td>(n=14)</td>
<td>(0.9-3.2)</td>
<td>(6.5-12.5)</td>
<td>(5.6-9.8)</td>
<td>(1.8-6.7)</td>
<td>(1.0-3.4)</td>
</tr>
<tr>
<td>Adult Females</td>
<td>2.1</td>
<td>7.9</td>
<td>6.9</td>
<td>5.2</td>
<td>2.0</td>
</tr>
<tr>
<td>(n=8)</td>
<td>(1.2-3.6)</td>
<td>(6.2-10.8)</td>
<td>(3.5-8.5)</td>
<td>(2.1-6.5)</td>
<td>(1.2-3.5)</td>
</tr>
<tr>
<td>Children (7-13 yrs)</td>
<td>2.4</td>
<td>9.5</td>
<td>8.9</td>
<td>5.3</td>
<td>1.8</td>
</tr>
<tr>
<td>(n=10)</td>
<td>(1.6-4.5)</td>
<td>(6.8-13.5)</td>
<td>(4.5-11.6)</td>
<td>(1.5-6.5)</td>
<td>(0.9-2.5)</td>
</tr>
</tbody>
</table>

*Geometric mean titers with range in parentheses.

Table 2. Comparison of neutralizing antibody titers in the first 3 months between the groups with and groups without passive immunization

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Neutralizing antibody titers (IU/mL) on days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Vaccine alone</td>
<td>1.8</td>
</tr>
<tr>
<td>(n=10)</td>
<td>(0.9-3.6)</td>
</tr>
<tr>
<td>HRIG+Vaccine</td>
<td>2.1</td>
</tr>
<tr>
<td>(n=12)</td>
<td>(0.8-3.9)</td>
</tr>
<tr>
<td>ERIG+Vaccine</td>
<td>1.9</td>
</tr>
<tr>
<td>(n=10)</td>
<td>(1.0-4.1)</td>
</tr>
</tbody>
</table>

*Geometric mean titers with range in parenthesis (P<0.005).

HRIG, Human rabies immunoglobulin.
ERIG, Equine rabies immunoglobulin.
The best indicator of protection following post-exposure immunization is the development of adequate titers of neutralizing antibodies. The role of cell-mediated immunity following rabies vaccination is still not very clear. The most significant aspect of the present study is the development of protective levels of antibodies by day 7 in all patients. The levels of neutralizing antibody titer obtained on day 7 are likely to be due to response to vaccine alone, as the titers were similar in those patients who took only vaccine without passive immunization. It is very unlikely that passively administered antibody would be still detectable on day 7. These patients had more than adequate titers throughout the study period, and the titers did not differ significantly between the groups with and without passive immunization. The multiple ID inoculation on different parts of the body did not in any way cause significant discomfort to these patients, except for slight pain at the inoculation sites. The side effects seen were mild, except in one patient who developed fever and enlargement of cervical lymph nodes.

Earlier studies from Thailand had revealed the efficacy of the two-site ID regimen in preventing rabies in people bitten by confirmed rabid dogs. In one of our previous studies, we have also shown the excellent immune response to this two-site ID regimen of PCEC vaccine. In another study, when we compared the titers obtained with two-site and eight-site regimens, seroconversion on day 7 was only in 20% of cases with the two-site regimen, in contrast to 100% seen with the eight-site regimen, and the titers of neutralizing antibodies were significantly greater on all days tested with the eight-site regimen (data to be published). Therefore, this makes the eight-site regimen more advantageous. In addition, this eight-site regimen requires fewer visits to the doctor. In all, our previous studies confirmed the efficacy of ID regimens in generating good and sustained immune responses. This encouraged us to try this regimen on patients bitten by confirmed rabid animals. We feel that the successful results seen in this study should encourage more such studies so that, in future, this cost-effective regimen can replace vaccination with highly reactogenic Semple vaccine, which is often responsible for severe side effects and vaccine failures.

ACKNOWLEDGEMENTS

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REFERENCES


