Comparative Evaluation of Immunogenicity, Reactogenicity and Safety of Purified Chick Embryo Cell Rabies Vaccine and Neural Tissue Rabies Vaccine

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Abstract
Objectives: 1. To compare the protective antibody titres on day 14, 30 and 90 after giving intramuscular (IM) injections of PCECV and subcutaneous injections of Nervous Tissue Vaccine. 2. To compare the immunogenicity and safety of PCECV and NTV.

Methods and Materials: The study enrolled cases in three groups. Group ‘C’ or control group: (n=38): This group comprised of 38 normal healthy volunteers without dog-bite. Group ‘A’ (n=102): This group included cases of dog-bite fulfilling inclusion/exclusion criteria. Each one of Group A and C were given PCECV as post exposure treatment (PET) on day 0-3-7-14-30 and 90. Group ‘B’ (n= 50): This group included 50 cases of dog-bite who received NTV. The rabies virus neutralizing antibody titres were estimated by RFFIT (Rapid Fluorescent Focus Inhibition Test) on day 0, 14, 30 and 90 days. 45 recipients of PCECV were re-tested for persistence of Protective Antibodies at the end of 1 year.

Results: Of these 37, 91 and 45 cases were evaluable in Groups C, A and B respectively. The antibody titres in Groups A, B, C were 13.4, 3.2, 22.8 IU/ml respectively; the protective titre being 0.5 IU/ml. 5% PCECV recepients had delayed response on day 30. 14% of NTV recepients did not seroconvert.

Conclusions: The Immunogenicity, Reactogenicity and safety of PCECV is well established. 5% of PCECV recepients showed a delayed sero conversion. 14% of NTV recepients did not sero convert at all. Therefore it is desirable to estimate antibody titres on day 14 after vaccination. If difficult, then all the cases of animal bite must receive passive immunization with rabies immunoglobulins.

INTRODUCTION
More than a million people take post-exposure rabies treatment in India every year, a 2/3rd of them are still vaccinated with Semple vaccine. Semple vaccine is a nervous tissue vaccine, although cheapest it has a very high rate of non-compliance and complications. It is not recommend by WHO because of its very short immune protection as well as its serious demyelinating complications. Annually, 30,000 rabies deaths are reported to occur in India, 96% of which are due to dog bites. This is just the tip of the ice-burg as a large number of cases go undiagnosed. As such rabies is not a notifiable disease in India.

Many factors like illiteracy, ignorance, poverty, non-availability of health care facilities, all contribute to the largest number of animal bite cases, not to seek medical care and post-exposure treatment. We had four cases of rabies in the past few years who developed rabies inspite of neural tissue rubies vaccination (NTV). Probably it was because the cases had taken incomplete course of treatment.

Neuroparalytic side effects of NTV like convulsions, transverse myelitis are well known. In order to reduce the morbidity and severe side effects of NTV, newer, safer vaccines have been developed.

The newer vaccines have rarely been compared with the currently used NTV. The high death rate of rabies in our country and the side effects of the NTV inspired us to undertake this comparative study of the immunogenicity, reactogenicity and safety of the beta propionolactone inactivated vaccine (NTV) with the purified chick embryo cell vaccine (PCECV), a tissue culture vaccine.

The aims and objectives of this study were as follows: To compare the protective antibody titres on day 14, 30 and 90
after giving intramuscular (IM) injections of PCECV and subcutaneous injections of nervous tissue vaccine and to compare the immunogenicity and safety of PCECV and NTV.

**MATERIAL AND METHODS**

**Study Design**

With the approval of the institutional ethical review committee, the study was conducted in the medicine department as per GCP guidelines.

All the males and females above the age of 12 years in a position to give written informed consent were included in the study.

Cases with history of previous antirabies vaccination, those reporting 24 hours after the animal bite, suffering from acute or chronic infectious disease, patients who had received antimalarials, steroids, immunosuppressive agents, phenytoin or anti-inflammatory drugs during the last two months, patients suffering from autoimmune disorders, alcoholics or IV drug abusers, pregnant females, patients allergic to study medications or chemically related substances, patients with psychiatric disorders were excluded from the study.

The study enrolled cases in three groups.

1. **Group A (n = 102)**: This group included cases of dog-bite fulfilling inclusion/exclusion criteria. Each one was given PCECV as post-exposure treatment (PET) on day 0, 3, 7, 14, 30 and 90.
2. **Group B (n = 50)**: This group included 50 cases of dog-bite who received NTV. The vaccine was given subcutaneously on anterior abdominal wall on day 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 irrespective of site of bite.
3. **Group C or control group (n=38)**: This group comprised of 38 normal healthy volunteers without dog-bite, the group mainly consisted of people at risk such as animal handlers, veterinary doctors, health care workers including doctors, nurses and other paramedical staff. In order to compare the immunogenicity, all these volunteers received simulated post-exposure prophylaxis (PEP) with PCECV. Each one received 1 ml of inj. PCECV on deltoid region on day 0, 3, 7, 14, 30 and 90.

Ten ml of blood from each case was drawn for estimation of antibody titres on day 0, 14, 30, 90. The serum was separated and stored at a temperature of -20°C, till the titres were estimated by RFFIT at a WHO approved centre in Kansas University, USA.

On subsequent visits, each patient was questioned regarding any adverse events in the form of fever, myalgias, pain at the site of injection, erythema, induration, headache, hypersensitivity reactions.

Forty five recipients of PCECV were re-tested for persistence of protective antibodies at the end of one year.

RFFIT is rapid fluorescent focus inhibition test for determining rabies virus neutralizing antibody titres. Estimation was carried out of all the samples at the end of the study.

1. The test sera is heated at 56°C, for 30 minutes before testing in order to inactivate complement. The positive serum control standard is diluted to a potency of 0.5 IU/ml and negative serum control standard is diluted to a potency of less than 0.1 IU/ml.
2. Screening dilutions of 1:5 and 1:50 are used for evaluation of vaccine efficacy.
3. The virus neutralizing antibody titres are calculated at the dilution factor of the highest serum dilution at which 50% of observed microscopic fields contain one or more infected cells.

**RESULTS AND DISCUSSIONS**

**Table 1 : Age and Sex distribution**

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Group C Males</th>
<th>Group C Females</th>
<th>Group A Males</th>
<th>Group A Females</th>
<th>Group B Males</th>
<th>Group B Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-20</td>
<td>0</td>
<td>0</td>
<td>31</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>21-30</td>
<td>9</td>
<td>7</td>
<td>34</td>
<td>0</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>31-40</td>
<td>9</td>
<td>6</td>
<td>13</td>
<td>2</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>41-50</td>
<td>0</td>
<td>1</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>51-60</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>61-70</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>16</td>
<td>93</td>
<td>9</td>
<td>38</td>
<td>12</td>
</tr>
<tr>
<td>Grand total</td>
<td>38</td>
<td>102</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The male predominance is seen due to the fact that most of the males had outdoor jobs or had to travel at odd hours and hence were exposed more to the dog bite as compared to females. Also in a male dominated society like ours, primarily the male patients seek medical attention as compared to females.

In order to compare immunogenicity, it was necessary to measure the antibody titres on day 0 as double check. Ab titres more than 0.5 IU/ml are the protective titres as per WHO guidelines. Table 2 shows the serostatus on day 0.

**Table 2 : Serostatus On day 0**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of recruited subjects</th>
<th>Ab. Titres &gt; 0.5 IU/ml</th>
<th>Evaluable subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>38</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td>A</td>
<td>102</td>
<td>1</td>
<td>101</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>5</td>
<td>45</td>
</tr>
</tbody>
</table>

In this study the control group did not show any Ab on day 0, but in other two groups six cases were rabies Ab positive on day 0. Although exclusion criteria were strictly applied, these six cases showing Ab on day 0 had to be excluded from final evaluation. Therefore, Table 2 shows the number of evaluable cases in the last column. A person not exposed to anti-rabies vaccine or previous dog-bite will not have anti-rabies antibodies on day 0, but in India, many dog-bite cases are not compliant with the full course of anti-rabies vaccine. Therefore, it is possible that they may not remember about the history of anti-rabies vaccination. Second possibility could be a sub-clinical exposure to rabies virus in...
the form of licking by a stray or a pet dog, which might not be remembered by the patient. There is one reference in literature which shows that 6% of veterinarians have protective antibodies against rabies due to subclinical exposures.

Table 3: Seroconversion On day 14

<table>
<thead>
<tr>
<th>Group</th>
<th>No of recruited subjects</th>
<th>Drop outs</th>
<th>Evaluable</th>
<th>Ab titres &gt; 0.5 IU/ml</th>
<th>% sero converted</th>
<th>Ab titres &lt; 0.05 IU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>38</td>
<td>1</td>
<td>37</td>
<td>37</td>
<td>100%</td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>102</td>
<td>10</td>
<td>91</td>
<td>86</td>
<td>94.5%</td>
<td>5</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>0</td>
<td>45</td>
<td>36</td>
<td>80%</td>
<td>9</td>
</tr>
</tbody>
</table>

This table 3 shows the sero conversion with protective antibody titres for rabies on day 14.

One volunteer dropped out from the study due to side effects like severe myalgia. In group B, 10 cases failed to follow the vaccination schedule, and hence these cases were excluded from final evaluation of protective antibody titres.

Seroconversion as per WHO recommendation i.e. antibody titre > 0.5 IU/ml had occurred in 100% of the healthy volunteers (Group C).

In Group A of post-exposure treatment with PCECV, out of 91 evaluable cases 86 had seroconverted. The rate of seroconversion on day 14 was 94.5%.

In group B, 36 out of 45 evaluable cases had seroconverted, the rate of seroconversion on day 14 was only 80%.

Table 4: Delayed seroconversion

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Day 30</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Further evaluation of non-converter with estimation of Ab titres on day 30 and 90 revealed (Table 4) that in group A, four out of five cases sero-converted on day 30. One patient showed a delayed sero-convension on day 90. The patient who had delayed sero-convension had oral candidiasis and hence was tested for HIV infection which revealed that the patient was HIV infected. A delayed sero-convension is known in presence of HIV infection.

As against, in group B with NTV, only three out of nine cases had sero-converted on day 30, while the remaining six didnot convert even at 90 days.

Table 5 compares the geometric mean titres (GMT) on day 14 in the three study groups. The antibody response to NTV is significantly (p < 0.0001) lower compared to PCECV. PCECV treated volunteers and PCECV recipients of dog-bite cases showed 45.6 fold and 27 fold respectively, higher titres of rabies IgG compared to NTV group (Fig. 1).

Table 6 shows that the adverse drug reactions were higher in the NTV group. No major side effects such as neuroparalytic complaints or convulsions were noted during the three months of study period.
point is seroconversion. The confirmation of seroconversion is usually not carried out. However, this study has revealed that 14% of NTV recipients did not seroconvert at all. Such a situation is risky because the patients get a pseudoconfidence of protection and the history of vaccination poses a diagnostic dilemma to the physician. It is evident from this study that, the estimation of antibody titres on day 14 of all the vaccines is desirable. If it is not possible then all the cases of dog-bite, in addition to wound toilet must receive passive immunisation with rabies immunoglobulins.

The antibody levels gradually fall due to process of decay of antibodies in the body. The estimation of antibody titres at the end of one year in the PCECV group revealed that all the 45 cases had antibodies > 0.5 IU/ml. High levels of antibodies maintained over a period of 1 year truly gives the protection against rabies. WHO always recommends the use of cell culture antirabies vaccine and has given up NTV long ago.

CONCLUSIONS

The immunogenicity, reactogenicity and safety of PCECV is well established. 5% of PCECV recipients showed a delayed sero-conversion. 14% of NTV recipients did not seroconvert at all. Therefore it is desirable to estimate antibody titres on day 14 after vaccination. If difficult, then all the cases of animal bite must receive passive immunization with rabies immunoglobulins. The community as well as medical fraternity must be made aware that in case of animal bite, wound toilet has no substitute, passive immunisation is crucial and human rabies immunoglobulins must be given.

Acknowledgements

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REFERENCES

6. WHO TRS No. 1984;709:28-34.