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A comparative study on the safety and immunogenicity of purified duck embryo cell vaccine (PDEV, Vaxirab) with purified chick embryo cell vaccine (PCEC, Rabipur) and purifed vero cell rabies vaccine (PVRV, Verorab)

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ABSTRACT

Rabies is a fatal but preventable disease. Cell culture vaccines (CCV) and purified duck embryo vaccines (PDEV) are currently recommended by WHO for post-exposure prophylaxis. In India, a PDEV (Vaxirab) is being manufactured and is in use since 2003. In the present study, we have evaluated the safety, immunogenicity and tolerance of this vaccine with two other WHO approved CCVs, viz., purified chick embryo cell vaccine (PCEC, Rabipur) and purified vero cell rabies vaccine (PVRV, Veroroab). This study was an open label, randomized phase IV comparative clinical trial. A total of 152 people bitten by dogs and other animals were recruited from 4 different centres from India. They were randomly assigned to receive one of the vaccines by Essen intramuscular regimen (52 subjects received Vaxirab and 50 each Rabipur and Verorab) and rabies immunoglobulin was also administered in all category III exposures. Their blood samples were collected on day 0 (prior to vaccination), 14, 28, 90 and 180. Side effects if any were monitored. The rabies neutralizing antibody titers in their blood samples were estimated by the rapid fluorescent focus inhibition test (RFFIT). Subjects in all three groups had neutralizing antibody titers by day 14 (>0.5 IU/mL) and geometric mean titers (GMT) observed for different vaccines on all days tested did not vary significantly ($p > 0.5$). Side effects observed were minimal and did not vary significantly among the groups. The results of the present study indicate that PDEV (Vaxirab) is as safe, tolerable and immunogenic as both PCEC (Rabipur) and PVRV (Verorab). Thus this vaccine can be a good alternative to WHO approved CCVs for rabies post-exposure prophylaxis.

1. Introduction

Rabies is a fatal but preventable disease. It is still a significant health problem in India and other developing countries despite the availability of safe and potent vaccines and rabies immunoglobulins (RIGs). As per a WHO estimate, world wide about 50,000 people die of rabies each year mostly in Asia, Africa and South America [1]. A recent WHO sponsored multicentric survey conducted by the Association for Prevention and Control of rabies in India (APCRI) revealed that about 20,000 people die of rabies every year in India and an astounding 17 million people are bitten by dogs and other animals [2]. Thus there is a great demand for rabies vaccines, and following the complete stopping of production and use of nerve tissue derived Semple vaccine from 2004, even government run antirabies clinics are administering modern rabies vaccines. Two types of modern vaccines are approved by WHO for post-exposure prophylaxis, viz., cell culture vaccines (CCV) and purified duck embryo vaccine (PDEV) [3]. Two WHO approved CCVs, viz., PCEC (Rabipur) and PVRV (Verorab) are available in India for the past 2 decades. The PDEV was developed by Gluck et al. [4] in 1985 and was originally manufactured by Berna Biotech, Switzerland under

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the brand name of Lyssavac N. The vaccine was found to be safe and effective by regular intramuscular regimen (Essen) and also was found to be effective by intradermal route [5–8]. The vaccine is reported to contain high content of rabies nucleoprotein (N) which is known to play a role in inducing protective immune response [5]. Following technology transfer, the same PDEV is now being manufactured in India by the Zydus Cadila Health Care Ltd. and marketed as Vaxirab. The technology transfer involved training of competent people, supervision of every step by the parent company while the manufacturing process was ongoing and after a rigorous check on the quality of the Indian product, a final approval was issued by the parent company. Subsequently the product was also tested by the Indian national quality control authority and the product was approved for use in India. The vaccine was also approved by WHO and included as a WHO approved vaccine in the recent WHO consultation on rabies.

A previous study conducted in India has shown that the indigenously produced PDEV (Vaxirab) was as good as the original PDEV (Lyssavac N) in terms of safety, immunogenicity and tolerance [9]. Presently the production of PDEV has been discontinued by the Berna Biotech and the PDEV manufactured in India (Vaxirab) is also being exported to some Asian countries under the brand name of Lyssavac N. The aim of the present study is to compare the safety, immunogenicity and tolerance of the indigenously produced PDEV (Vaxirab) with two other WHO approved vaccines, viz., PCEC (Rabipur) and PVRV (Verorab) which are available not only in India but also in the international market.

2. Materials and methods

2.1. Subjects

One hundred and fifty two people exposed to rabies through dog and other animal bites were recruited for the study at four different centres. All subjects had single bites belonging to WHO category II or III [10]. None had multiple bites or bites on head and neck. The rabid status of the biting animal was not confirmed in any case as all were street dogs and were not traceable after the incident. The centers were: Kempegowda Institute of Medical Sciences (KIMS), Bangalore, Institute of Preventive Medicine (IPM), Hyderabad both located in southern part of India, MKCG Medical College, Behrampur, Orissa located in the eastern part of the country and Grant Medical College, Mumbai located in the western part of the country. A total of 152 subjects were recruited and they were allocated to 3 groups randomly to receive one of the study vaccines. There were 52 subjects in the Vaxirab group and 50 each in Rabipur and Verorab group. The study was approved by the human ethics committee of all the four institutions and written consent was taken from each subject either in English or local language.

The inclusion criteria were willingness to participate in the study, to be in the age group of 5–55 years, willing to give blood samples on stipulated dates and available for follow-up for 6 months post-vaccination. The exclusion criteria were history of previous rabies vaccination or any animal bite, pregnancy, concomitant illness or on any medication, simultaneous participation in any other study and subjects known to be HIV positive. The study was started simultaneously at all centers and conducted for a period of one year from 2006 to 2007. The data was collected systematically in a standard report format.

2.2. Vaccines

The vaccines used were PDEV (Vaxirab) produced and marketed by Zydus Health Care Ltd., Ahmedabad (batch no. RG 110, potency 8.0 IU/dose), PCEC (Rabipur, produced by Chiron vaccines, India and marketed by Sanofi Aventis, Mumbai, India, batch no. A0 826, potency 8.5 IU/dose) and PVRV (Verorab, manufactured by Sanofi Pasteur, France and marketed by Ranbaxy, Mumbai with potency 13 IU/dose).

Subjects with category II were administered with one dose (1 mL in case of Vaxirab and Rabipur and 0.5 mL in case of Verorab) of vaccine intramuscularly (IM) on days 0, 3, 7, 14 and 28. Subjects with category III exposures also received equine rabies immunoglobulin (ERIG, Zyrig batch no. U 7001, potency 300 IU/mL) as per WHO recommendations.

2.3. Estimation of rabies virus neutralizing antibody titers (RVNA)

About 5 mL of venous blood was collected from each subject on day 0 (before vaccination) and on days 14, 28, 90 and 180 following vaccination. The serum was separated and stored at −20 °C before transporting in dry ice to the Department of Neurovirology, National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore which is a WHO collaborating center for reference and research on rabies.

The serum samples were tested for RVNA by rapid fluorescent focus inhibition test (RFFIT) at the above centre. The methodology used was as recommended by WHO with some modifications [11]. The cell line used was BHK 21 (ATCC CCL10) and the challenge virus used was CVS 11 adapted to grow in BHK 21 cells. The test was performed using 96-well tissue culture plates (Nunc, USA). The dose of the CVS (obtained from Central Research Institute, Kasauli, India as a mouse brain homogenate and adapted to grow in BHK 21 cells) used was 50 FFD_{50}. The plates were read using Nikon inverted fluorescence microscope (Eclipse TS 100). The 50% neutralizing titers of the serum samples were converted to international units in comparison to titers obtained with a in-house reference rabies immunoglobulin (RIG) calibrated against 2nd international reference RIG procured from National Institute of Biologicals, UK and having a potency of 30 IU/mL. This product was obtained through WHO.

2.4. Adverse reactions

All subjects were closely interrogated and physically examined on each of their visit for any adverse reaction to the vaccines. Reactions if any were recorded.

2.5. Statistical analysis

The geometric mean titers (GMT) of the antibody titers were calculated along with geometric standard deviation (GSD), standard error (SE) and 95% confidence interval. Analysis of variance (ANOVA) was applied to know the difference in GMTs on different days. The difference between proportions of adverse reactions among the groups were tested using Z test.

3. Results

The age and sex distribution, category of bites and animals involved in each vaccine group is depicted in Table 1. There were 122 males and 30 females recruited in the study. The biting animal was dog in 133 cases (87%) followed by monkey in 12 cases (8%) and cats in 7 cases (5%). There were 104 category III cases and 48 category II cases. Vaxirab was administered in 52 cases, Rabipur in 50 and Verorab in 50 cases. The RVNA response observed at different time points in the 3 groups is depicted in Table 2. It can be seen that all the subjects had more than adequate titers of antibodies (>0.5 IU/mL) by day 14 which persisted on days 28–180. Maximum titers were observed on day 28 in all groups. The difference in titers observed between the 3 groups was not statistically