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Immunogenicity and safety study of Indirab: A Vero cell based chromatographically purified human rabies vaccine

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ABSTRACT

A chromatographically purified Vero cell rabies vaccine, Indirab manufactured by Bharat Biotech International Limited, Hyderabad, India was subjected to safety and immunogenicity studies by both intramuscular and intradermal routes of administration in parallel with a reference vaccine, Verorab. A Pre-exposure study was undertaken in 239 subjects by intramuscular (IM) route (Study I), Post-exposure study in 188 patients by intramuscular route (Study II) and Simulated post-exposure study in 134 subjects by intradermal (ID) route (Study III). All subjects in these studies were administered with either the test or the reference vaccine as per WHO approved intramuscular and intradermal regimens. The blood samples were collected on days 0, 14 and 35 in case of Study 1, and days 0, 14, 28 and 90 in case of studies II and III. In all studies both vaccine groups had adequate antibody titers (>0.5 IU/mL) on all days tested post-vaccination and there was no significant difference in the titers observed ($p > 0.05$). Some side effects like pain, induration, itching and fever were noted in both vaccine groups in all studies. Both vaccines were well tolerated. Hence it can be concluded that Indirab is as safe and immunogenic as Verorab when administered by both intramuscular and intradermal routes.

1. Introduction

Rabies is a fatal viral encephalitis which still continues to be a major public health and veterinary problem in many parts of the world particularly the developing nations of Asia and Africa. As per a WHO estimate around 55,000 people die of rabies every year globally [1]. India reports the highest number of human rabies deaths and animal bites. As per a recent WHO sponsored multi-centric study conducted in India by the Association for Prevention and Control of Rabies in India (APCRI), about 20,000 human rabies deaths are reported and around 17 million animal bites occur annually [2]. Till recently all the animal bite victims who attended government run antirabies clinics and hospitals were administered with nerve tissue derived Semple vaccine. Use of cell culture vaccines (CCVs) were restricted to a small segment of population who could afford their cost. However following the order of Supreme Court of India, the use of Semple vaccine was completely banned in 2005 and all the animal bite victims attending the government run clinics are also being administered with CCVs. Consequently the demand for CCVs has increased considerably over the past few years. Modern CCVs such as Purified Chick Embryo Cell (PCEC) vaccine and Purified Vero cell Rabies Vaccine (PVRV) are all being manufactured in India in the private and public sectors for many years but the quantity produced is not sufficient to meet the required demand. Thus there is scope for increasing the production capacities of these vaccines and also produce newer vaccines in sufficient quantities to meet the huge demand for rabies vaccine in this country. In this context the production and supply of a new PVRV (Indirab) which is derived from Pitman-Moore strain and purified chromatographically is a welcome step. The other brands of PVRV produced in India and France are presently purified by zonal centrifugation. Efforts were made earlier by Lang et al. [3] to purify PVRV by chromatographic method, and a few batches were tested for potency and immunogenicity by pre-exposure vaccination of healthy volunteers. A post-exposure clinical trial was also conducted in Philippines using this CPVRV with good results

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[4]. However, commercialization of this product was not materialized and presently Indirab is the only chromatographically purified PVRV available commercially in the world. This vaccine is produced in India by Bharat Biotech International Ltd., Hyderabad using disposable cell factories with automated cell factory manipulator (ACFM) and WHO approved current Good Manufacturing Practices (cGMP). The vaccine was certified for human use by the Drugs Controller General of India (DCGI) after undergoing clinical trials in 3 phases, viz. Pre-exposure studies in healthy volunteers, Post-exposure studies by standard intramuscular route and Simulated post-exposure studies by intradermal routes. A WHO approved vaccine viz. Purified Vero cell Rabies Vaccine (PVRV, Verorab) was used as reference vaccine to evaluate this new vaccine in terms of safety, tolerability and immunogenicity. This paper gives an overview of these clinical trials. The results of these trials clearly indicate that this new chromatographically purified rabies vaccine produced indigenously is safe, well tolerated and as immunogenic as the reference vaccine used in these studies.

2. Materials and methods

The protocols of these clinical studies were approved by Drugs Controller General of India (DCGI) and Institutional Ethics Committee (IEC) of the respective study centers. Written informed consent was obtained from all the subjects before enrollment into the respective study.

2.1. Study design

All these were blinded, randomized, active controlled, multicenter, comparative (parallel), Phase III studies. These studies were performed under out-patient department conditions in subjects enrolled as per the inclusion and exclusion criteria. The inclusion criteria were healthy subjects, not previously exposed to rabies and not taken any rabies vaccine in the past, willingness to participate in the study and their availability for the entire study period in Study 1 and Study 3. The Study 1 (Pre-exposure intramuscular) was conducted in four centers, Study 2 (Post-exposure intramuscular) in two centers and Study 3 (Simulated post-exposure Intradermal) in two centers. The test and reference vaccines in intradermal (ID) studies were administered by trained staff from Institute of Preventive Medicine (IPM), Hyderabad, India and Kempegowda Institute of Medical Sciences (KIMS), Bangalore, India under the supervision of Principal Investigators. In each center, the subjects received either the test vaccine or the reference vaccine as per the randomization codes generated. A commercially available WHO pre-qualified vaccine Verorab procured from open market was used in all studies as a reference vaccine. Subjects were randomly assigned to one of the groups as per the codes generated by the computer programme Minitab 14 version. The group assignment was not known to the subject, investigator and the laboratory staff performing the serologic assay.

2.1.1. Study 1 (Pre-exposure intramuscular)

A total of 239 healthy volunteers participated in the study; 180 were given Indirab vaccine (Batch No. 62AN4001, potency 5.84 IU/dose) and 59 subjects were given Verorab vaccine (Batch No. Y0659, potency >2.5 IU/dose). Both test and reference vaccines were reconstituted with 0.5 ml diluent provided along with the vaccine vial. Vaccine was administered intramuscularly on days 0, 7 and 28 and blood samples of 5 ml were collected on days 0, 28 and 35. We were able to collect blood samples at the end of 1 year from 18 subjects who had taken Indirab and 12 subjects who had taken Verorab. The study population was between 10 and 65 years of age. This broad age range was particularly included to assess the safety and immunogenicity in young children as well as elderly people.

2.1.2. Study 2 (Post-exposure Intramuscular)

A total of 188 (aged 5–55 years) cases participated in the study, out of which 141 subjects were given Indirab vaccine (Batch No. 62AN4002, potency 6.08 IU/dose) and 47 subjects were given Verorab vaccine (Batch No. Z0502, potency >2.5 IU/dose). Majority of animal bite victims in India belong to this age group. Category III patients were administered rabies immunoglobulin (Equine rabies Immunoglobulin, Equirab, Bharat Serums and Vaccines) as per WHO recommended dose. Both test and reference vaccines were reconstituted with 0.5 ml diluent. Vaccine was administered intramuscularly on days 0, 3, 7, 14 and 28 and blood samples of 5 ml were collected on days 0, 14, 28 and 90.

2.1.3. Study 3 (Simulated post-exposure intradermal)

A total of 134 healthy volunteers (aged 18–55 years) participated in the study, 68 were given Indirab vaccine (Batch No. 62AN7005, potency 5.7 IU/dose) and 66 subjects were given Verorab vaccine (Batch No. A0920, potency >2.5 IU/dose). Both test and reference vaccines were reconstituted with 0.5 ml diluent. Vaccine was administered ID as per updated Thai Red Cross Regimen (2-2-2-0-2) on days 0, 3, 7 and 28 and blood samples of 5 ml were collected on day 0, 14, 28 and 90.

2.2. Estimation of Rabies Virus Neutralizing Antibody (RVNA) titers

The blood samples were centrifuged after collection, serum separated and transferred into 2 coded cryovial aliquots and stored at −20 °C until transferring to testing laboratory, Department of Neurovirology, National Institute of Mental Health and Neurosciences (NIMHANS) Bangalore, India which is WHO collaborating centre for reference and research on rabies. Serum samples were tested by Rapid-Fluorescent-Focus Inhibition Test (RFFIT) as per WHO recommendation with some modifications [5]. The test was performed using 96 well tissue culture plates (Nunc, USA) and the cell line used was BHK 21 (ATCC CCL 10). The virus used was CVS 11 (obtained from Central Research Institute, Kasauli, India) and adapted to grow in BHK 21. We used 50 FFD_{50} of the virus as challenge dose. The highest dilution of the serum samples which reduced fluorescent foci in 50% of the cells was taken as the endpoint. The titers were expressed in IU/mL in comparison to an in-house reference serum calibrated against 2nd international reference serum having a potency of 30 IU/mL. This was procured from National Institute of Biological Standards, UK.

2.2.1. Statistical analysis

Geometric mean titers (GMT) and 95% confidence intervals (CI) were computed by taking the exponent (log 10) of the mean and of the lower and upper limits of the 95% confidence intervals of log 10 transformed titers. The safety end points (percentage of subjects and 95% confidence intervals) were solicited and unsolicited adverse events. We used Mann-Whitney Test to compare the values between the vaccine groups. A p value 0.04 or less was considered to indicate statistical significance. Data analysis was done by using MINITAB software (version 14).

3. Results

The demographic profile of the subjects recruited in the 3 studies is given in Table 1. The purpose of the Study I was to assess the safety and immunogenicity of this new vaccine in healthy volunteers. It can be seen from Table 2 that all subjects in both vaccine groups attained more than adequate levels of RVNA titers by day