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Safety and immunogenicity of a new purified vero cell rabies vaccine (PVRV) administered by intramuscular and intradermal routes in healthy volunteers

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ABSTRACT

Background: Rabies is 100% fatal but preventable with modern vaccines and immunoglobulins. There is a huge demand for rabies vaccines in developing countries of Asia and Africa. We have developed a new purified vero cell rabies vaccine (PVRV) and evaluated its safety and immunogenicity in healthy volunteers by intramuscular (IM) and intradermal (ID) routes of vaccination.
Methodology: Sixty adults aged between 18 and 50 years were recruited in this actively controlled Phase I clinical study and were randomized to receive three 1 ml or 0.1 ml doses of new PVRV intramuscularly or intradermally on days 0, 7 and 21. The control group received commercially available PVRV (Verorab) by intramuscular route. Adverse events (AEs) were recorded with diary cards till day 28 post-vaccination. Immunogenicity was assessed on day 0, 7, 21 and 42 by rapid fluorescence focus inhibition test (RFFIT).
Results: In all, 116 solicited local and systemic events were reported across the three groups. Most were mild and resolved without sequelae. Also the few unsolicited events, deemed unrelated to the study vaccines, caused no problems. No significant changes in the routine laboratory parameters were found. Two doses of a vaccine elicited protective titres (≥0.5 IU/ml) in all subjects, the GMTs varying between 0.57 and 0.69 IU/ml on day 7, 3.07 and 3.97 IU/ml on day 21, and 6.12 and 8.52 IU/ml on day 42 post-vaccination.
Conclusions: PVRV was well tolerated and showed good immunogenicity regardless of whether administered intramuscularly or, using a tenth of that volume, intradermally. Further studies with this new vaccine are warranted.

1. Introduction

Rabies is a fatal viral zoonotic disease that occurs at least in 100 countries and territories, mostly in the developing world. More than 3.3 billion people are living at risk of this severe infection [1], and estimated 55,000 deaths occur per year of which 20,000 in India alone and 24,000 in Africa (an incidence of 2 or 4/100,000 population at risk, respectively) [2]. Even these figures might be underestimates [3].

Limited access to vaccines is one of the reasons for this high mortality. As per an estimate, globally, ≥15 million people receive rabies prophylaxis annually [1], however, in India alone; there are >17 million animal bites annually, and the majority of the victims reside in the rural areas and are often unvaccinated [4].

High costs of the traditional rabies vaccines as well as availability pose a continuing problem in poor countries and no doubt contribute to the low coverage of vaccination. For years, safe and efficacious vaccines have been produced in cell cultures such as those derived from human diploid cell, purified vero cell, or chick embryo cells. All these vaccines have been used successfully in pre- and post-exposure prophylaxis [5]. Being very immunogenic, the cell culture vaccines (CCVs) can also be administered intradermally (ID) which is a major advantage, as the dose is only a fraction (0.1 ml) that used in the traditional intramuscular (IM) route, thereby reducing the cost of active immunization [3]. The ID administration is now routinely used even in the post-exposure prophylaxis in countries such as India, the Philippines, Sri Lanka and Thailand [6,7].

Using the vero cell line, Serum Institute of India, Ltd. (SIIL), Pune, has developed its own rabies vaccine which is intended for both intramuscular and intradermal routes. After thorough single- and repeated-dose studies in rats and mice which had shown its good tolerability and non-teratogenicity, a Phase I clinical trial was

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considered justified. We here report the first results of this new vaccine in human volunteers.

2. Subjects and methods

2.1. Study design

A phase I open-label, randomized study was conducted at the King Edward Memorial Hospital Research Centre, Pune, India, after approval from the Institutional Ethics Committee (IEC) and the Drugs Controller General of India. Prior to screening an informed consent was required in writing. The study was carried out in accordance with Schedule Y of the Drugs and Cosmetics Act of India (2005), Indian Council of Medical Research's Ethical Guidelines for Biomedical Research on Human Participants (2006) and the International Conference on Harmonization (ICH) Good Clinical Practices Guidelines (1996). It was also registered in the clinical trials registry of India (CTRI/2011/07/001903).

The participants, enrolled between September 2011 and October 2011, were healthy adults of both gender aged between 18 and 50 years. The exclusion criteria were prior receipt of any rabies immunoglobulin and/or rabies vaccine; history of potential rabies exposure; treatment with an immunosuppressant; acute febrile, infectious, or chronic disease; breast feeding; seropositivity for HIV, hepatitis B or C; and any vaccination or receipt of a serum or blood product within the previous 30 days.

The participants were divided in three groups with a 1:1:1 randomization. Group I and Group II received SIIL PVRV in a volume of 1 ml injection intramuscularly or 0.1 ml intradermally, on days 0,7 and 21, whereas Group III was immunized with 0.5 ml of a control vaccine, Verorab® (Sanofi Pasteur) intramuscularly. Appearance of a papule causing a peaud' orange (orange peel) confirmed correct ID administration.

Simple block randomization was used to allocate subjects throughout the enrolment period, and sealed envelopes with detailed instructions were used for each subject.

2.2. Study vaccines

SIIL PVRV is a lyophilized vaccine (Batch number 148E0001, expiry date April 2012) containing inactivated purified rabies antigen (Pitman Moore, PM3218 as virus strain) produced using Vero ATCC CCL 81 cells. The diluent (sterile water for injection, batch number 065I0001; expiry date Feb 2015) was provided in a separate 1 ml vial. After reconstitution, a single dose 1 ml contained inactivated, purified rabies antigen (not less than 2.5 IU), glycine (40 mg), sucrose (40 mg) and human serum albumin (25% 10 mg). The potency of the batch was 4.35 IU/dose.

The production process of SIIL PVRV starts with revival of vero ATCC CCL81 cells. The revived cells are given serial passages to seed the cell cube system. The cells in cell cube system are then infected with working seed virus, followed by the cell cube system washed with virus medium. After washing, multiple harvesting is carried out at 48–72 h interval. Each harvest is clarified and concentrated. The concentrated virus harvest is inactivated by ß propiolactone. The inactivated rabies antigen is purified using column chromatography. The rabies antigen eluted from the column is dialyzed and then stabilized with sucrose–glycine to get final purified antigen.

Verorab® (Sanofi Pasteur, France, batch number E0997-3, expiry date September 2012) with diluent (batch no. E5884-2, expiry date September 2012) was used as the control vaccine. In this case, a single dose of the freeze-dried vaccine (WISTAR strain RABIES PM/WI 38-1503-3 M) had been produced on vero cell line, and contained at least 2.5 IU of inactivated rabies antigen, maltose and human plasma albumin. The diluent consisted of 4% sodium chloride in a volume of 0.5 ml.

2.3. Assessment of immunogenicity and safety

Serum samples were collected prior to vaccination on day 0 and on day 7, 21 and 42 post vaccination. The sera were tested for rabies virus neutralizing antibody by rapid fluorescence focus inhibition test (RFFIT) at the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore. The test used BHK 21 (ATCC CCL 10) and 96 well tissue culture plates (Sigma) and BHK21 adapted CVS 13 strain of rabies virus. The reference serum used was an in house serum calibrated against 2nd international reference standard having a titre of 30 IU/ml (obtained from the National Institute of Biological standards, UK). Briefly, doubling dilutions of serum samples and reference serum (after heat inactivation at 56 °C for 30 min in a water bath) in duplicate were made in 96 well plates using IMDM (Sigma Cat No. 17633). To each 100 μl of serum dilution 100 μl of CVS (100 FFD_{50}) was added and the plate was incubated at 37 °C for 1 h. A confluent monolayer of BHK 21 cells were trypsinized and re- suspended in 10 ml of IMDM with 10% FCS (Sigma, cat No. F2442). Cell control and virus controls were also included. To each well of the 96 well Plate 100 μl of cell suspension was added and the plate was incubated at 37° C in a CO_2 incubator (Sanyo, Japan). After 24 h the cells were fixed in cold acetone for 30 min and stained by direct FAT using commercially available rabies N conjugate (Light diagnostics USA, Cat No. F199). The plates were then observed under an inverted fluorescence microscope (Nikon Eclipse). The highest dilution of serum showing 50% inhibition of fluorescence foci was taken as end point dilution. The titre was converted to IU/ml in comparison with reference serum.

The safety was assessed by medical history, vital sign measurements, and routine laboratory tests for haematology, chemistry and urinalysis. These data were collected on day 7 and 28, whereas the solicited local and systemic events were captured with structured diaries during 7 days after each vaccination. Unsolicited adverse events (AE) and serious adverse events (SAEs) were captured till day 42 from the first vaccination. Potential concomitant medications were recorded on all the visits. Those abnormal clinical laboratory test values which were assessed as clinically significant were recorded as an AE.

2.4. Statistical methods

Since this was a Phase I study, the sample size was not determined from power analysis. Intention-to-treat (ITT) population was used for safety analysis, while per protocol (PP) population was used for immunogenicity. All analyses were performed using SAS® (9.2) and model test assumptions were examined graphically and analytically. The proportion of subjects with RVNA titres $\geq$ 0.5 IU/ml was compared between groups by two sided fisher's exact test. GMTs between Study Groups were compared by Kruskal–Wallis test.

3. Results

3.1. General

Of the 98 screened subjects, 60 eligible subjects (52 males and 8 females) were enrolled and randomized into the three arms. The varied between 19 and 48 years across all three groups (Table 1). In Group I, one vaccinee received the third dose one day too early, and another subject received dose 3 ID. These