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An immunogenicity study of a newly introduced purified vero cell rabies vaccine (*Abhayrab*) manufactured in India

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Abstract

Purified Vero cell culture rabies vaccine "*Abhayrab*" manufactured by Human Biologicals Institute, Ooty, India was subjected for immunogenicity studies. Pre-exposure study was undertaken on 60 healthy volunteers (Group I) with vaccination on days 0, 7 and 21. A group of 75 patients of category II (Group II), 67 of category III (Group III) were given post-exposure prophylaxis and 88 patients of category III were administered with rabies immunoglobulins (Group IV) along with post-exposure prophylaxis as per World Health Organization (WHO) recommendations with a booster on day 90. The volunteers and patients vaccinated showed very few adverse side effects. The blood samples collected from volunteers (Group I) on days 14, 35 and 365 and patients (Group II–IV) on days 14, 30, 90 and 365 showed geometric mean titres (GMT) of >0.5 IU/ml. The study indicated new rabies vaccine manufactured in India was found to be safe and immunogenic.

Keywords: Post-exposure rabies treatment; Rabies tissue culture vaccine; Rabies in India

1. Introduction

Rabies is endemic in India where approximately 5,000,000 persons undergo post-exposure rabies vaccination annually. The exact number of human rabies deaths is not known since rabies is not a reportable disease in India. Estimates range from 15,000 to 30,000 deaths annually [1]. Sheep brain derived Semple type rabies vaccine is still being manufactured and utilized for the majority of exposed patients in India, even though this vaccine has been discouraged by the World Health Organization (WHO) [2]. The high cost of tissue culture vaccine and inertia have been the main barrier to the replacement of Semple vaccine by WHO recommended post-exposure rabies prophylaxis (PEP), utilizing tissue culture derived products [3]. Fortunately, Indian manufacturers have now rallied to the challenge and several "home grown" tissue culture rabies vaccines are appearing on the potentially large public and private sector Indian market. Vero cell culture based Rabies vaccine *Abhayrab*, manufactured by the Human Biologicals Institute (HBI) at Ooty, is to the best of our knowledge, the first new product which has undergone an independent immunogenicity study.

The Vero cell line was first described by Yasumura in 1962. This resilient high-yield permanent cell line is derived from *Cercopithecus aethrops* (African Green Monkey) kidney cells. It has a long and successful history of being used in production of rabies and polio vaccines worldwide [4]. Introduction of a Vero cell rabies vaccine into Thailand, along with the economical intradermal Thai Red Cross post-exposure vaccine schedule, was instrumental in abolishing the use of animal brain tissue derived rabies vaccines by the late 1980s in that country. Vero cell derived rabies vaccines are now being manufactured in France, Columbia, China and India. *Abhayrab* is grown on a Vero cell line utilizing the L. Pasteur 2061 strain of rabies virus. It is inactivated with β *pro-*

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piolactone (BPL), lyophilized and reconstituted in 0.5 ml of physiological saline. It is manufactured under good manufacturing practices (GMP) and fulfills WHO recommendations for potency. It was licensed by the Drugs Controller General of India (DCGI) in 1999 and has been marketed throughout the country since that time. Before the initiation of the current trial, a simulated post-exposure prophylaxis study was done in 40 healthy volunteers. They were administered "*Abhayrab*" intramuscularly into the deltoid muscle on days 0, 3, 7, 14 and 30. Estimation of Rabies neutralizing antibody titers revealed geometric mean titres (GMT) >0.5 IU/ml in all individuals on days 14, 30, 90 and 365 (unpublished data).

The study site was the Rabies Clinic of the Institute of Preventive Medicine, Hyderabad. It is a State government operated facility independent from the manufacturer of *Abhayrab*. The regular medical and nursing staff of this facility were the designers and investigators for this project. The Rabies clinic of this institute treats over 4000 patients for potential rabies exposures monthly. Approximately, 20–25 human rabies cases are also diagnosed annually. Estimates by local public health authorities are that approximately 5% of dog bite victims reporting for treatment have been bitten by rabid animals. The vast majority of patients receive Semple vaccine at no charge. Only a small proportion (1–2%) of Class III exposures are also given rabies immunoglobulin. This is due to the lack of funding and the fact that patients have to pay for this product which the majority cannot afford and also due to the fact that RIGs are not available throughout the year.

2. Materials and methods

The clinical study was approved by the Institutional Ethics Committee (IEC) of the Institute of Preventive Medicine, Hyderabad. The IEC took into consideration the fact that the vaccine was evaluated in non-exposed healthy individuals prior to being used in the present study.

Informed written consent was obtained from all the patients before enrolment in the study.

Study subjects were divided into four groups as follows:

Group I consisted of 60 healthy volunteers aged 20–56 years (mean 41) and with a male/female ratio of 52:8. They were given a course of pre-exposure rabies vaccine using *Abhayrab* 0.5 ml (one ampoule) intramuscularly into deltoid muscle on days 0, 7 and 21 (Table 1).

Group II patients consisted of 75 subjects with WHO Category II injuries [2] (mostly bites). They received *Abhayrab* 0.5 ml intramuscularly into deltoid muscle on days 0, 3, 7, 14, 30 and 90 without immunoglobulin.

Group III patients consisted of 67 subjects with WHO Category III injuries [2] (mostly bites) who received the same regimen as Group II and also without rabies immunoglobulin.

Group IV patients consisted of 88 subjects with WHO Category III injuries (multiple, facial and hand bites) [2] who also received equine rabies immune globulin (ERIG) 40 IU/Kg which was injected into and around bite wounds as recommended by WHO.

It is not known how many of the patients had actually been the victims of rabid animals since these were not available for testing.

The *Abhayrab* vaccine used was batch AYB 26/99, potency of 10.82 IU per 0.5 ml dose. The ERIG used was manufactured by Pasteur Merieux (Imorab) with a concentration of 200 IU/ml (Batch No. T5060-4).

Blood was collected for neutralizing antibody determination from all patients on days 0, 14, 30, 90 and 365. The sera of the volunteers, who received pre-exposure vaccination (Group I), were tested on days 0, 14, 35 and 365. Neutralizing antibodies were determined using coded duplicate samples and the Rapid Fluorescent Focus Inhibition Test (RFFIT) [5] at the Pasteur Institute of India, Coonoor. Results were decoded in the presence of Dr. A.M. Ghanekar by Dr. Suhasini V. Reddy.

Compliance among subjects in Group I (the volunteers) for the full course and blood collection was 35%. That for Groups II–IV was 65%, 52% and 64% respectively. None of the patients in Groups II–IV died of rabies by the end of the second year of follow up. Adverse side effects were mild to minimal and consistent with previous studies of other WHO recognized tissue culture rabies vaccines [6–14].

Antibody titers were above the WHO recommended protective level (0.5 IU/ml) [2] on days 14, 30, 90 and 365 (Table 1) except for one patient, a 33 year old healthy man who had a borderline low titer (0.46 IU/ml) on day 14. He had received ERIG and was found to have a titer of 2.00, 4.82 and 1.55 by days 30, 90 and 365, respectively.

3. Results and discussion

All Group I volunteers demonstrated a lasting immune response that was well above the 0.5 IU/ml WHO mandated minimum level on days 14, 35 and 365 (Table 1). Potentially rabies exposed patients in Groups II, III (without ERIG) and IV (with ERIG) also demonstrated neutralizing antibody titers above the 0.5 IU/ml with the exception of one patient in Group IV. He had a lower titer on day 14 which rose to an acceptable levels by days 30, 90 and 365. It is noteworthy that geometric mean antibody titers (GMT) were 7.04, 9.47 and 8.42 IU/ml in Groups II–IV, respectively, by day 90 prior to the administration of the booster vaccine injection. The day 90 booster was administered as a safety measure since antibody titers were not yet known to the investigators at that time. It appears that the 90 day booster dose will not be required for *Abhayrab*. It had been used with other new tissue culture rabies vaccines in Europe and the Americas when they were first introduced but was later abandoned [15].

Adverse side effects were few and comparable or fewer to those seen with other tissue culture rabies vaccines [6–14].

Table 1
Serum neutralizing antibodies (RFFIT) in volunteers and patients bitten by dogs vaccinated with *Abhayrab*

Day	Group I	Group II	Group III	Group IV
0				
GMT	<0.05	<0.05	<0.05	<0.05
n	60	75	67	88
Range	–	–	–	–
n <0.5	60	75	67	88
7				
GMT	–	0.83	0.78	0.61
n	–	75	67	88
Range	–	<0.05–8.00	<0.05–4.20	<0.05–4.20
n <0.5	–	61	53	81
14				
GMT	12.69	13.53	15.78	10.34
n	56	72	65	82
Range	0.53–64.00	1.00–66.51	1.00–66.51	0.46–66.51
n <0.5	0	0	0	1
35(Group I), 30(Group II–IV)				
GMT	18.19	15.03	8.04	11.66
n	52	66	62	81
Range	2–32	2.10–64.00	1.50–32.00	2.00–64.00
n <0.5	0	0	0	0
90				
GMT	–	7.04	9.47	8.42
n	–	48	39	59
Range	–	1.60–33.33	1.05–56.51	1.05–66.51
n <0.5	–	0	0	0
365				
GMT	12.26	4.15	7.82	5.80
n	21	49	35	56
Range	0.56–32.00	1.05–31.11	1.05–66.51	0.92–66.51
n <0.5	0	0	0	0

The cut-off point for the RFFIT test was 0.05 IU/mL. *n* <0.5 are the number of subjects with antibody titers under 0.5 IU/mL. Vaccination schedule: Group I: days 0, 7 and 21; Group II–IV: 0, 3, 7, 14, 30 and 90.

They consisted of minor irritations (0.2%) or mild pain at injection sites (4.06%). Only very few patients complained of systemic symptoms such as fatigue (0.33%), dizziness (0.06%), headache (0.13%) or mild fever (0.67%). All recovered with symptomatic treatment only (analgesics and antihistamines) and without having to abandon or alter the PET regimen. Among the group of 88 patients who also received ERIG on day 0, only one had an early local injection – site reactions (erythema and pruritis starting within 1 or 2 days). One subject had a mild serum sickness-like illness starting on day 8. Both resolved with symptomatic treatment; demonstrating again the safety of highly purified equine rabies immune globulin [16]. We conclude that *Abhayrab*, a new rabies vaccine developed in India, is a highly immunogenic and safe product.

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