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Safety and immunogenicity study of a new purified chick embryo cell rabies vaccine Vaxirab-N (Pitman–Moore strain) manufactured in India

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Zydus Cadila Health care, India developed a new purified chick embryo cell rabies vaccine (PCECV, Vaxirab-N; 1 mL) by adapting Pitman–Moore strain of virus on to the chick embryo fibroblast cell line in 2006. During 2007–10, a series of safety and immunogenicity studies were conducted as per ICH-GCP guidelines after obtaining permission from Drug Controller General of India. In the first study, Vaxirab-N was administered to 35 healthy adult volunteers by intramuscular (IM) route using pre exposure regimen. The geometric mean concentration (GMC) of rabies virus neutralizing antibody (RvnAb) of 7.5 IU/mL on day 35. In the second study, Vaxirab-N was administered to 35 healthy adult volunteers using simulated post- exposure prophylaxis regimen by IM route. A GMC of 6.3 IU/mL on day 14, 13.2 IU/mL on day 28 and 8.6 IU/mL on day 90 was obtained. In the third study, Vaxirab-N administered by intradermal (ID) route using Updated Thai Red Cross (TRC) regimen in 36 healthy adult volunteers showed GMC of 7.8 IU/mL on day 14, 11.5 IU/mL on day 28 and 6.0 IU/mL on day 90. The 4th study was multi centric and Vaxirab-N was administered to 129 animal bite cases by IM route using post-exposure Essen regimen. The GMC following this schedule was 8.2 IU/mL on day 14, 13.01 IU/mL on day 28, 7.92 IU/mL on day 90 and 3.72 IU/mL on day 180. Mild to moderate adverse events were reported to Vaxirab-N but no serious adverse events were reported in any of these studies. In conclusion, Vaxirab-N developed by Zydus Cadila was found to be safe and immunogenic by both intramuscular and intradermal route and is recommended for rabies prophylaxis (CTRI No. 2010/091/000055 and 2010/091/000509).

Introduction

Rabies is a fatal viral encephalitis transmitted to man from the bite of rabid animals. Globally, an estimated 55 000 persons die of rabies every year of which 31 000 (56%) die in Asia and 24 000 (44%) in Africa.[1] Nearly 3.3 billion people are having potential threat of human infection mainly in Asia and Africa. An estimated 20 000 human rabies deaths occur in India every year.[2] Nearly 17 million animal bite cases occur in India every year and in 96% of cases dog is the main biting animal.[3] Rabies can be prevented by timely initiation of post-exposure prophylaxis (PEP) which includes proper local treatment of bite wounds, administration of rabies vaccines either by intramuscular (IM) or intradermal (ID) route and local infiltration of rabies immunoglobulins (RIG).[4] The demand for potent and safe cell culture vaccine is increasing in most Asian and African countries. The first human rabies vaccine to be marketed was the human diploid cell vaccine developed by Wiktor and Koprowsky.[5] The first primary cell culture vaccine for human was produced by Barth et al. in 1985 using chick embryo fibroblasts and Flury LEP strain of rabies virus.[6] This purified chick embryo cell vaccine (PCECV) has a long record of good safety and immunogenicity.[7,8] Another PCEC vaccine (Kaketsuken rabies vaccine) was produced in Japan and found to have good immunogenicity and safety record by both

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IM and ID routes of vaccination.[9,10] However, the Japanese vaccine is not widely used in other parts of the world. In the past two decades several new rabies vaccines derived from verocell, a continuous cell line were developed and these purified verocell rabies vaccines (PVRV) have been found to have good safety and immunogenicity and are comparable to HDCV, PCECV, and PDEV.[11-13] However the advantage of diploid and primary cell culture vaccines over vaccines derived from continuous cells is the absence of potentially gene transforming or tumorigenic substrate DNA because of extensive purification procedure. However, verocell lines have been used extensively for rabies and polio vaccine production for the past 30 y. Vero-based rabies vaccines are licensed in Europe, while Vero-based polio vaccine is licensed in US as well as in Europe. Vero-based rotavirus and influenza vaccines also are in development.

In recent times Zydus Cadila health care Ltd, India developed a PCEC vaccine (Vaxirab-N) by using the Pitman-Moore strain of rabies virus cultivated in primary chick fibroblast cells to produce highly safe and immunogenic rabies vaccine. The vaccine has been produced in the state of the art manufacturing plant located at Ahmedabad, India using stringent current good manufacturing practices (cGMP) and in process quality control. It was licensed for use by Drug Controller General of India (DCGI), the national drug authority in 2012. The process of manufacturing this new PCEC vaccine (Vaxirab-N) using Pitman-Moore strain has been patented.[14]

During year 2008–10, a series of safety and immunogenicity phase 2 and 3 clinical studies with Vaxirab-N were conducted in India as per ICH-GCP guidelines. We report here the details of the clinical studies including safety and immunogenicity of this new PCEC vaccine.

Results

Study on healthy volunteers (phase 2)

Pre-exposure study

The mean age of males was 33.7 ± 11.1 y and of females was 28.6 ± 7.7 y; males were 14 (41.2%) and females were 20 (58.8%). Nineteen (55.9%) subjects were graduates by education and 21 (61.8%) were skilled workers by occupation. One (2.9%) subject experienced "mild pain" at the site of vaccine administration which resolved spontaneously after 4 d. No serious/severe/unexpected adverse event was reported by any of the subjects and none of the subjects discontinued the study due to adverse event. The geometric mean concentration (GMC) of rabies virus neutralizing antibody (RvnAb) titers is presented in **Table 1**. All the subjects had adequate and protective titers of ≥0.5 IU/mL on day 35.

Table 1. RvnAb response to Vaxirab-N administered using Pre exposure prophylaxis by IM route (study 1)

Days	n	Range	GMC (IU/mL)	GSD (IU/mL)	95% confidence interval	
					Lower bound	Upper bound
35	34	3.5 to 10.5	7.5	1.4	6.74	8.29

There were no demonstrable RvnAb titers on day 0 in all study subjects. IU, international standard; GMC, geometric mean concentration; GSD, geometric standard deviation.

Table 2. RvnAb response to Vaxirab-N administered using simulated post exposure prophylaxis by IM route (study 2)

Days	n	Range	GMC (IU/mL)	GSD (IU/mL)	95% confidence interval	
					Lower bound	Upper bound
14	34	3.5 to 10.5	6.3	1.4	5.64	7.02
28	34	7.5 to18.6	13.2	1.3	12.5	14.24
90	34	3.8 to 12.6	8.6	1.4	7.77	9.60

There were no demonstrable RvnAb titers on day 0 in all study subjects. IU, international standard; GMC, geometric mean concentration; GSD, geometric standard deviation.

Simulated post-exposure prophylaxis using IM route

The mean age of males was 27.5 ± 6.8 y and of females was 27.1 ± 8.4 y; males were 13 (38.2%) and females were 21 (61.8%). Twenty-five (73.5%) subjects were graduates by education and 16 (47.1%) were professional workers by occupation. None of the subjects experienced any adverse event of whatsoever nature during the entire study period. The geometric mean concentration (GMC) of rabies virus neutralizing antibody (RvnAb) titers from day 14 till day 90 is presented in **Table 2**. All the subjects had adequate and protective titers of ≥0.5 IU/mL from day 14 till day 90.

Simulated post-exposure prophylaxis using ID route

The mean age of males was 28.42 ± 7.98 y and of females was 24.70 ± 5.01 y; males were 19 (52.8%) and females 17 (47.2%). Eighteen (94.7%) of males educated up to high school and postgraduate courses; 14 (82.4%) of females had studied up to intermediate. A total of 20 mild adverse events were reported by 7 (19.4%) out of 36 subjects. The incidence of adverse drug events was 6.9%. The adverse events were induration (2.4%), itching (2.1%) at the site of vaccination, fever (1.1%), redness at injection site (0.7%), myalgia (0.3%) and loose stools (0.3%). No serious/severe/unexpected adverse events reported by any of the subjects enrolled in the study. The geometric mean concentration (GMC) of rabies virus neutralizing antibody (RvnAb) titers from day 14 till day 90 is presented in **Table 3**. All the subjects had adequate and protective titers of ≥0.5 IU/mL from day 14 till day 90.

Post exposure study using IM route (phase 3 or pre-licensing study)

The mean age of males was 32.56 ± 10.87 and of females was 31.07 ± 10.15; males were 101 (78.3%) and females 28 (21.7%). Majority 124 (96.1%) had dog bites. 87 (67.4%) were category III exposures and all of them received Equine rabies immunoglobulin (ERIG) as per WHO recommendation. Two subjects were lost to follow up due to migration (**Table 4**).

Table 3. RvnAb response to Vaxirab-N administered using updated TRC regimen in healthy volunteers (study 3)

Days	n	Range	GMC IU/mL	GSD IU/mL	95% confidence interval	
					Lower bound	Upper bound
14	35	4.5 to 12.4	7.81	1.27	7.19	8.49
28	36	7.5 to 19.5	11.60	1.27	10.70	12.57
90	35	4.3 to 7.9	6.03	1.26	5.57	6.54

There were no demonstrable RvnAb titers on day 0 in all study subjects. IU, international standard; GMC, geometric mean concentration; GSD, geometric standard deviation.

A total of 23 mild adverse events were reported by 18 (14.2%) out of 127 patients. The adverse events were fever (1.1%), headache (0.9%), local pain at site of vaccination (0.6%), body ache (0.3%), giddiness (0.3%), rashes (0.2%), and swelling of the face (0.2%). No serious/severe adverse event was reported by any of the patients enrolled in the study. The geometric mean concentration (GMC) of rabies virus neutralizing antibody (RvnAb) titers is presented in **Table 5**. All the subjects had adequate and protective titers of ≥0.5 IU/mL from day 14 till day 180 as per WHO recommendation. Both groups of subjects with or without ERIG had protective titers from day 14 through day 180. There was no significant decrease in antibody response after ERIG administration (**Table 6**).

Discussion

In this study we have determined the safety and immunogenicity of a new PCEC vaccine (Vaxirab-N) manufactured for the first time in India. The vaccine differs from the currently available PCECV with regard to the strain of the virus i.e Flury LEP strain. The Pitman-Moore strain of the virus used in this new version of PCECV is well documented for safety and immunogenicity and has been genetically characterized and found to have 100% homology with original Pasteur virus strain.[15] The first ever cell culture vaccine for human use, the human diploid cell vaccine (HDCV) was produced using Pitman–Moore strain. Flury LEP strain was used by Barth et al. for the development of the first PCEC vaccine (Rabipur) as the virus strain was already adapted to grow in chick embryo. As Rabipur was the first human vaccine produced using a "non-Pasteur" virus strain, initially there was some concern about its efficacy. In fact, when the immunogenicity studies of Rabipur were being done, there was a suggestion to use the vaccine strain of the virus itself rather than the regularly used CVS strain. Indeed some earlier studies have shown that Rabipur produced greater RvnAb response and greater protection in animal experiments when homologous virus was used as a challenge virus.[16,17] In spite of these observations, Rabipur has been found to be efficacious in preventing rabies in exposed individuals both by conventional IM and the ID routes of vaccination

This new PCEC vaccine (Vaxirab-N) has been produced after successful adaptation of Pitman–Moore strain of rabies virus. The genetic stability of the strain was maintained during the adaptation procedure. Also recent studies based on genetic characterization of different vaccine strains have shown close homology between Pasteur and Pitman–Moore strains.[18]

This newly produced vaccine has been evaluated for safety and immunogenicity following pre-exposure vaccination and post-exposure administration by conventional intramuscular route and also by simulated post-exposure regimen using updated Thai Red Cross intradermal regimen. In all the trials, the subjects had excellent immune response and vaccine was found to be safe. The rabies virus neutralizing antibody titers obtained by subjects from day 14 onwards was much greater than the required level of 0.5 IU/mL and more than adequate antibody titers were present on day 180. There were minimal adverse events which subsided eventually without the need for any additional medication. The good immunogenicity of this vaccine by ID route is particularly encouraging as many people exposed to animals in India are now being administered vaccination by ID route. The safety and immunogenicity results of Vaxirab-N is comparable to HDCV, original PCECV (Rabipur) and PVRV. At present we cannot comment on the efficacy of vaccine in the post-exposure group as the rabid status of biting animal was not confirmed in any case. However, efficacy studies are now rarely done because of cost and other logistical issues and proof of good immunogenicity based on well controlled clinical studies is considered sufficient to prove the efficacy of vaccine. The unit price of Vaxirab-N is comparable to other cell culture rabies vaccines in Indian market.

The strength of this study is that safety and immunogenicity of Vaxirab-N, a newly produced PCECV with Pitman–Moore strain

Table 4. Post-exposure prophylaxis IM route (study 4): details of subjects, category of exposures and ERIG administration

Parameter		Number (%)
Age (mean ± SD) in years	Male	32.56 ± 10.87
	Female	31.07 ± 10.15
Sex	Male	101 (78.3)
	Female	28 (21.7)
Category of exposure	II	42 (32.6)
	III	87 (67.4)
Biting animal	Dog	124 (96.1)
	Monkey	04 (3.1)
	Cat	01 (0.8)
ERIG	Administered	87 (67.4)*

SD, standard deviation; ERIG: equine rabies immunoglobulin. *2 subjects who had Category III exposure and received ERIG were lost to follow up.

Table 5. RvnAb response to Vaxirab-N administered in animal bite cases using Essen regimen (study 4)

Days	n	Range	GMC IU/mL	GSD IU/mL	95% confidence interval	
					Lower bound	Upper bound
14	127	4.5 to 12.8	8.20	1.27	7.86	8.54
28	127	7.6 to16.9	13.01	1.20	12.61	13.43
90	101	4.5 to 12.5	7.92	1.30	7.52	8.34
180	68	1.4 to 7.5	3.72	1.53	3.36	4.12

There were no demonstrable RvnAb titers on day 0 in all study subjects. IU, international standard; GMC, geometric mean concentration; GSD, geometric standard deviation

Table 6. RVNA titers on different days in subjects administered vaccine with or without RIGs

Days	ERIG	N	Mean	SD	*t* value	*P* value*
Day 14	Without ERIG	42	8.46	1.31	0.3777	0.7063
	With ERIG	85	8.37	1.24		
Day 28	Without ERIG	42	12.9	1.24	1.3772	0.1709
	With ERIG	85	13.21	1.17		
Day 90	Without ERIG	35	7.33	1.32	0.2587	0.7964
	With ERIG	66	7.26	1.28		
Day 180	Without ERIG	16	3.86	1.62	0.2081	0.8358
	With ERIG	52	3.95	1.48		

**P* value not significant

of rabies virus has been studied using standard IM regimen with pre-exposure vaccination, simulated post exposure prophylaxis, suspect rabies exposed individuals and also with updated TRC regimen in healthy volunteers. Based on the results of the study the vaccine has been approved by the regulatory authorities of Government of India for both pre-exposure and post-exposure prophylaxis by intramuscular and intradermal routes. However, some of the limitations of the study were small sample size, single arm, non-randomized and non-comparative study. These issues are being addressed in another ongoing study.

To conclude, India is highly endemic for rabies and has the largest number of dog bites in the world and the demand for a potent and safe vaccine is ever increasing. The study demonstrated that Vaxirab-N developed by Zydus Cadila was safe and immunogenic when used by Essen IM or updated TRC intradermal route with or without rabies immunoglobulin. However, this new vaccine needs to be evaluated with one or more vaccines with long standing safety and immunogenicity record in post exposure situation in a larger population.

Materials and Methods

Permission to conduct pre-registration phase 2 and 3 trials was obtained from Drug Controller General of India (DCGI). These studies were also registered with clinical trial registry of India (CTRI) of Indian Council of Medical Research (ICMR). During a period of 3 y (2007–2010), a total of 4 studies were conducted after approval of institutional ethics committee. Subjects were enrolled after explaining the nature and possible consequences of the studies and obtaining signed informed consent. The standard inclusion and exclusion criteria were followed for conduct of these studies (**Table** 7). The sample size in these studies was calculated as per Indian drug regulatory authority requirements. Vaccine used in these studies was Vaxirab-N (purified chick embryo cell vaccine, lyophilized, reconstituted with 1 mL diluent).

Pre-exposure study by intramuscular (IM) route (study 1), simulated post-exposure study by IM route (study 2), and simulated post-exposure study by intradermal (ID) route (study 3) were conducted at anti-rabies clinic of Kempegowda Institute of Medical Sciences (KIMS) Hospital and Research center, Bangalore, India and a post-exposure study in animal bite/exposures (study 4) was conducted at 3 centers viz. Kempegowda Institute of Medical Sciences (KIMS) Hospital and Research Center, Bangalore, Institute of Preventive Medicine (IPM), Hyderabad, and MKCG Medical College Hospital, Berhampur.

Studies on healthy volunteers (phase 2)

Study 1 (pre-exposure intramuscular)

The safety and immunogenicity of Vaxirab-N was studied in 35 healthy volunteers using pre- exposure vaccination regimen. Vaxirab-N (vaccine lot: CG 101; potency: 7.72 IU/IM dose) was administered intramuscularly into the deltoid muscle on days 0, 7, and 28. One subject was lost to follow- up due to migration. The sera were analyzed for RvnAb on days 0 and 35.

Study 2 (simulated post-exposure intramuscular)

Subsequent to completion and availability of results of first study, a study was undertaken to assess the safety and immunogenicity of Vaxirab-N administered in 35 healthy volunteers using simulated post-exposure regimen. The

Table 7. Inclusion and exclusion criteria used in the studies

	Inclusion criteria		Exclusion criteria
1	Human subjects of either gender between 18–55 y age.	1	History of animal bite in the past
2	Subjects likely to be available for all visits during follow-up period.	2	Pregnancy and lactation
3	Subjects willing to sign Informed consent.	3	Subjects have received any type of rabies vaccination in the past.
		4	Subjects have received any type of rabies immunoglobulin (human/equine) in the past.
		5	Subjects suffering from any other illness of whatsoever nature.
		6	Subjects are on steroids or any other immunosuppressant or known to be HIV positive.
		7	Subjects are on concomitant antimalarials.
		8	Subjects with history of allergy to any ingredient of the vaccine.
		9	Participation in another clinical trial in the past 3 mo

Vaxirab-N (vaccine lot: CH 101; potency: 7.04 IU/IM dose) was administered intramuscularly into the deltoid muscle on days 0, 3, 7, 14, and 28. One subject was lost to follow- up due to migration. The sera were analyzed for RvnAb on days 0, 14, 28, and 90.

Study 3 (Simulated post exposure intradermal)

To comply with the national requirements for an effective rabies vaccine for intradermal use another study was conducted to assess the safety and immunogenicity of Vaxirab-N administered intradermally using simulated post-exposure prophylaxis in 36 healthy volunteers. Each subject was given 0.1 ml of Vaxirab-N (vaccine lot: CH 101; potency: 7.04 IU/IM dose) intradermally per site and on two such ID sites per visit on days 0, 3, 7, and 28 using Updated Thai Red Cross (2–2-2–0-2) regimen. The sera were analyzed for RvnAb on days 0, 14, 28, and 90.

Post exposure studies using intramuscular route (IM) (Phase-3 or pre licensing study)

Study 4

A multi-centric study was undertaken to assess the safety and immunogenicity of Vaxirab-N administered using post-exposure prophylaxis schedule in 129 animal bite cases. Adult patients of either sex having category II or III animal bite (as per WHO classification) were enrolled into the study after following inclusion and exclusion criteria. The animal bite cases were administered Vaxirab-N (vaccine lot: CH 101; potency: 7.04 IU/IM dose) intramuscularly into the deltoid muscle on days 0, 3, 7, 14, and 28. All the patients with category III exposures also received equine rabies immunoglobulin (ERIG; Equirab, potency 300 IU/mL) after skin sensitivity test in a dose of 40 IU/kg body weight as per WHO recommendation. Two subjects were lost to follow- up due to migration. The sera were analyzed for RvnAb on days 0, 14, 28, 90, and 180.

Adverse reactions

Subjects were observed for 30 min after vaccine administration for possible immediate adverse reactions and also during subsequent follow-up visits by soliciting any adverse event and also by physical examination. Information on adverse events that may occur after last vaccination was obtained by telephonic contact. The reactions were graded on 4-point scale: 0, None (absence of symptoms), 1, Mild (presence of mild symptoms), 2, Moderate (symptoms which have an impact on normal activities), and 3, Severe (symptoms which prevent daily activities).

Estimation of rabies virus neutralizing antibody titers (RvnAb)

The immunogenicity of Vaxirab-N was assessed by estimating rabies virus neutralizing antibody titers (RvnAb) on coded sera samples using rapid fluorescent focus inhibition test (RFFIT) at WHO Collaborating Center for Reference and Research on Rabies, National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India. The test was done as per WHO recommended procedure with some modifications.[19] We 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 titer of 30 IU/mL (obtained from 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 to 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 plates 100 µl of cell suspension was added and the plate was incubated at 37 °C in a CO_2 incubator (Sanyo). 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). 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 titer was converted to IU/mL in comparison with reference serum.

Statistical analysis

The data were analyzed by computing range, geometric mean concentration (GMC), geometric standard deviation (GSD) and 95% confidence interval (CI) for GMC. To find the difference between antibody titers in subjects who were administered vaccine alone and vaccine plus RIG "student unpaired t test" was used.

Disclosure of Potential Conflicts of Interest

Shamanna Manjula and Pradip Maganlal Patel, two co-authors are employees of Zydus Cadila Health care Pvt. Ltd which manufactures the new PCEC vaccine (Vaxirab-N).

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