International Journal of Community Medicine and Public Health
Hardanahalli RS et al. Int J Community Med Public Health. 2018 Feb;5(2):585-589
http://www.ijcmph.com
pISSN 2394-6032 | eISSN 2394-6040

DOI: http://dx.doi.org/10.18203/2394-6040.ijcmph20180233

Original Research Article

Clinical evaluation of purified chick embryo cell rabies vaccine administered intradermally in animal exposures

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Received: 18 October 2017
Revised: 12 December 2017
Accepted: 30 December 2017

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ABSTRACT

Background: In India, presently there are two purified chick embryo cell culture vaccines (PCECV) viz., Rabipur (Flury LEP strain) and Vaxirab – N (Pitman Moore strain) which are commonly used both in public as well as private sectors. The present study was conducted to assess the clinical efficacy in terms of safety, immunogenicity and survival status of both the PCECV administered animal exposures taking complete PEP at the anti-rabies clinic.
Methods: A longitudinal study was conducted at the anti-rabies clinic, Kempegowda Institute of Medical Sciences (KIMS), Bangalore, India. 86 suspect rabid dog bite cases attending clinic were enrolled and followed up for 1year. All the animal bite cases were given post exposure prophylaxis of full course of PCECV i.e. Rabipur or Vaxirab - N as per schedule intradermally using updated Thai Red Cross regimen. The rabies virus neutralizing antibody (RVNA) concentrations on days 14, 28, 90, and 180 were tested by modified rapid fluorescent focus inhibition test.
Results: Out of 86 study subjects, 43 subjects received Rabipur and another 43 subjects received Vaxirab –N vaccines. The incidence of adverse drug events (ADEs) was found to be 9.3%. All subjects had protective RVNA titers of ≥0.5 IU/ml from day 14 till day 180. All the study subjects were healthy and alive after 6 months of completing PEP.
Conclusions: The currently available purified chick embryo cell culture rabies vaccines are safe, immunogenic and clinically effective for post exposure prophylaxis in animal bite cases, which will help in eliminating human rabies by 2020.

Keywords: PCECV, Rabipur, Vaxirab-N, Animal exposure

INTRODUCTION

Human rabies is a viral zoonotic disease that occurs in 150 countries and territories covering all the continents except Antarctica.[1] The disease that is practically 100% fatal, poses a potential threat to over 3.3 billion people worldwide.[2] In this regard, World Health Organization (WHO), Geneva in December, 2015 has called for elimination of dog-mediated human rabies by 2030.[3]

In India, animal bites is a major public health problem and an estimated 17.4 million bites occur annually and dog is the principal transmitter of the disease in 97% of these cases.[4] Therefore, as an rabies endemic country, we

must gear up to accomplish the task of elimination of dog-mediated human rabies at the national level.

Rabies is practically 100% preventable; timely and correct post exposure prophylaxis (PEP) to animal bite victims is life-saving. Proper wound management and simultaneous local infiltration of rabies immunoglobulin combined with anti-rabies vaccine is almost invariably effective in preventing rabies, even after high-risk exposure.[5]

Since their development, more than four decades ago, Cell culture vaccines (CCVs) have proved to be safe and effective in preventing rabies. These vaccines are intended for both pre- and post-exposure prophylaxis and have been administered to millions of people worldwide. But, the affordability to CCVs for intramuscular administration during PEP is a major constraint in developing countries of Asia and Africa. Therefore, World Health Organization (WHO) recommends intradermal route of vaccination with CCVs for these countries to reduce the quantity of vaccine and the cost of vaccination. Considering the large number of animal bite cases in the country and huge demand for CCVs, following the recommendations of WHO and ICMR, the drug controller general of India (DCGI) approved intra dermal administration of rabies vaccines using updated TRC regimen in 2006.

In India, presently there are two purified chick embryo cell culture vaccines (PCECV) are available, both of which are approved for intradermal vaccination viz., Rabipur (Flury LEP strain) and Vaxirab – N (Pitman Moore strain) and are commonly used both in public as well as private sectors. The present study was conducted to assess the clinical efficacy in terms of safety, immunogenicity and survival status of both the PCECV administered intra-dermally in animal exposures taking complete PEP at the anti-rabies clinic.

METHODS

A longitudinal study was conducted at the anti-rabies clinic, Kempegowda Institute of Medical Sciences (KIMS) Hospital and Research Centre, Bangalore, India for a period of 18 months from January 2014 to June 2015 after obtaining clearance from the institutional ethics committee. Eighty six suspect rabid dog bite cases attending anti rabies clinic during the first 6 months of the study period were included in the study after considering the inclusion and exclusion criteria and taking written informed consent.

Inclusion criteria

Inclusion criteria were subjects with category II and III bites; Subjects who were willing to participate in the study; 3) Subjects available for minimum of 6 months follow-up; 4) Subjects willing to give blood samples on recommended days.

Exclusion criteria

Exclusion criteria were subjects who had already started anti-rabies vaccination outside and come for rabies immunoglobulin administration; subjects who had already taken pre-exposure prophylaxis or come for re-exposure vaccination; subjects with history of allergy to any ingredient of the vaccine.

Post exposure prophylaxis including proper wound wash with soap and water, local infiltration of rabies immunoglobulin (RIGs) in all category III cases and administration of full course of PCECV ie., Rabipur or Vaxirab - N as per schedule was done. The vaccine was administered intradermally using Updated Thai Red Cross regimen i.e., 2 doses of 0.1 ml vaccine given intradermally over both the deltoid muscle on days 0, 3, 7 and 28.

A thorough and detailed enquiry was done among all the study subjects to rule out taking any rabies vaccine either as pre exposure prophylaxis (PrEP) or PEP and history of any animal bite in the past. Similarly, any concomitant medical conditions / treatments were ruled out.

A standard case record form was maintained for each bite victim that included details of socio-demographic profile, type of exposure, post exposure prophylaxis provided, adverse drug reactions (ADR) and treatment of ADRs at the study centre along with their telephone number for further follow up. At the first visit information was collected from the study subjects using a pre-tested structured questionnaire regarding socio-demographic characteristics, relevant past and present medical history, anthropometry, physical and systemic examination findings, details of PEP provided, adverse drug reactions (ADR), treatment of ADRs. The study subjects were followed up for 1year to know their survival status.

Assessment of safety

Following vaccination, all the subjects were observed for half an hour for possible immediate local/ systemic adverse drug events (ADEs). At the end of half an hour, reactogenicity was recorded, only if the subject spontaneously complained of a problem to a question on general wellbeing i.e., unaided recall. The subjects were given a follow up card to indicate if they had any late adverse events and was recorded in the subsequent visits i.e., on Day 3, 7 and 28.

Assessment of immunogenicity

Blood samples were drawn from all the subjects after taking informed consent, on days 0, 14, 28, 90, and 180 for estimation of rabies virus neutralizing antibody (RVNA) by modified rapid fluorescent focus inhibition test (RFFIT) at the Department of Neurovirology, National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India which is a WHO collaborating center for reference and research on rabies.

All the serum samples were analysed by using modified RFFIT test.

Estimation of rabies virus neutralizing antibody (RVNA)

Modified RFFIT was done as per WHO recommended procedure. The cell line used was BHK 21 (ATCC CCL 10) and 96 well tissue culture plates (Sigma) and BHK21 adapted CVS 11 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). 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 uL of serum dilution 100 uL 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 plate 100 uL 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, 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 titer was converted to IU/ml in comparison with reference serum.

All the study subjects were followed up for 6 months to know their survival status. All the biting dogs could not be traced or caught for laboratory examination due to logistical difficulties.

Statistical analysis

The data was analyzed statistically by computing percentages, geometric mean concentration (GMC), geometric standard deviation (GSD), standard error (SE), 95% confidence interval (CI) for GMC

RESULTS

Eighty-six study subjects with suspected rabid dog bite were included in the study. Out of them 43 subjects received Rabipur and another 43 subjects received Vaxirab –N vaccines. The socio- demographic profile of the study subjects is as follows:

Majority of the study subjects were in the age group of 18-25 years (31.3%) with the mean age being 34.16 ± 12.12 years. 58 (67.4%) were males and 28 (32.6%) were females. Most of the study subjects i.e., 23 (26.8%) had completed intermediate education and belonged to upper middle socio-economic status (41.9%) (Table 1).

Table 1: Socio demographic characteristics of the study subjects.

Socio demographic characteristics		Number (n=86)	Percentage (%)
Age (in years)	18-25	27	31.3
	26-35	23	26.8
	36-45	17	19.8
	46-55	19	22.1
Sex	Male	58	67.4
	Female	28	32.6
Education	Illiterate	10	11.7
	Primary school	05	5.8
	Middle school	07	8.2
	High school	19	22.0
	Intermediate	23	26.8
	Graduate/Postgraduate	19	22.0
	Professional degree	03	3.5
Socio-economic status*	Upper	02	2.4
	Upper middle	36	41.9
	Lower middle	27	31.3
	Upper lower	20	23.2
	Lower	01	1.2

* Modified Kuppuswamy Socioeconomic Status classification

In the present study, the biting animal was dog in all 86 (100%) subjects, out of which 45 (52.4%) were pet dogs and 41 (47.6%) were stray dogs. 72 (83.7%) of the animal bite victims had category III exposure and 14 (16.3%) had category II exposure. The site of bite was lower limb in 45 (52.4%) followed by upper limb (23.2%), multiple sites (11.6%), trunk (9.4%) and head and neck (3.4%). Most common type of wound was abrasion i.e., 62 (72.0%) followed by laceration (10.4%), puncture wounds (9.4%) and multiple wounds (8.2%) (Table 2).

Table 2: Details of exposure.

Details of exposure		Number (n=86)	Percentage (%)
Biting animal	Dog	86	100.0
Type of dog	Pet dog	45	52.4
	Stray dog	41	47.6
Category of exposure	III	72	83.7
	II	14	16.3
Site of bite	Head & neck	03	03.4
	Trunk	08	09.4
	Upper limb	20	23.2
	Lower limb	45	52.4
	Multiple site	10	11.6
Type of wounds	Abrasion	62	72.0
	Laceration	09	10.4
	Puncture wounds	08	09.4
	Mixed wounds	07	08.2

Table 3: Adverse drug events (ADEs).

Adverse drug event	Number (%)
Itching	26 (17.1)
Induration	06 (6.9)
Pain	06 (6.9)
Erythema	23 (26.7)
Fever	01 (1.1)
Myalgia	02 (2.3)
Total	64/688 (9.3)

Table 4: Immunogenicity of PCECV among study subjects.

Day of blood sample	No. of subjects	GMC (IU/ml)	GSD (IU/ml)	SE (GMC)	95% CI	
					Lower Bound	Upper Bound
14	85	13.50	1.20	0.19	13.24	13.76
28	84	11.57	1.15	0.18	11.32	11.82
90	80	9.71	1.15	0.16	9.46	9.96
180	78	8.19	1.17	0.15	7.93	8.45

The incidence of adverse drug events (ADEs) was found to be 9.3%. The common ADEs were erythema, itching, pain at the site of injection, induration, myalgia & fever. All the ADEs were mild and resolved without any complications (Table 3).

The GMC of RVNA titers were 13.50 IU/ml, 1157 IU/ml, 9.71 IU/ml and 8.19 IU/ml on days 14, 28, 90 and 180 respectively. All subjects had protective RVNA titers of ≥0.5 IU/ml from day 14 till day 180. All the study subjects were healthy and alive after 6 months of completing PEP (Table 4).

DISCUSSION

Intradermal administration of cell culture rabies vaccines offers an equally safe and immunogenic alternative to intramuscular rabies vaccination and requires less volume of vaccine and is recommended by WHO in resource constraint countries.[6] The present study showed that, currently used PCECVs Rabipur and Vaxirab – N when administered intradermally are safe and efficacious for post exposure prophylaxis.

The present study showed that, the incidence of ADEs was 9.3%. The other studies also showed similar results. A study conducted by Madhusudana et al, showed that the incidence of ADEs was 9.5% with the use of purified cell culture rabies vaccine (PCECV) and another study by Sudarshan et al, with PCECV using KIMS intradermal regimen also showed a total ADEs of 3.1.[7,8] Therefore, the safety of study vaccines was comparable to other studies and it confirms that available PCECV is safe for PEP among animal bite victims.

World Health Organization recommends a minimum RVNA titer of >0.5 IU/ml of serum for protection against rabies from day 14 onwards.[6] The objective of

vaccination in post-exposure prophylaxis (PEP) is to stimulate the immune system to produce antibody titers of at least 0.5 IU/ml by day 14, and persist for a long time in animal bite cases. In the present study, all animal bite victims had protective RVNA titers of >0.5 IU/ml on days 14, 28, 90 and 180 with the Geometric Mean Concentration of 13.50 IU/ml, 11.57 IU/ml, 9.71 IU/ml and 8.19 IU/ml respectively. Similarly, a study conducted by Khawplod et al showed GMC titers of 9.14IU/ml, 3.93 IU/ml, 3.59 IU/ml and 1.1 IU/ml on days 14, 28, 90 and 360 respectively.[9] Another study conducted by Suntharasamai et al, the GMC on days 14, 28, 90 and 180 were 9.07 IU/ml, 11.32 IU/ml, 3.64 IU/ml and 1.54 IU/ml respectively showing protective levels.[10] Similarly, a study conducted by Madhusudana et al using TRC regimen showed GMC of 4.3 IU/ml, 9.0 IU/ml, 6.7 IU/ml and 3.7 IU/ml on days 14, 30, 90 and 180 respectively.[11] The immunogenicity of the PCECV in the present study were similar to other studies and it confirms the immunogenicity of PCECV administered intradermally among animal bite victims.

CONCLUSION

The currently available purified chick embryo cell culture rabies vaccines are safe, immunogenic and clinically effective for post exposure prophylaxis in animal bite cases, which will help in eliminating human rabies by 2020.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Hardanahalli RS, Veena V, Holla R, Annadani RR, Sathish Chandra MR. Clinical evaluation of purified chick embryo cell rabies vaccine administered intradermally in animal exposures. Int J Community Med Public Health 2018;5:585-9.