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Original Research Article

Comparative evaluation for safety & potency of inactivated Cell Culture Rabies Vaccines from four Indian manufacturers

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Abstract

Rabies is a fatal but preventable disease. Various cell culture rabies vaccines (CCRV) are recommended by World Health Organization (WHO) for pre- exposure prophylaxis and post-exposure therapeutic application. In the present study, we have evaluated seventy batches of inactivated CCRV, for safety and potency by test for Virus Inactivation and National Institute of Health (NIH) potency test respectively in *Swiss albino* mice, produced by four Indian manufacturers, using different cell substrates, rabies virus strains and inactivation methods. For a single batch evaluation, 0.03ml of undiluted vaccine sample was injected intracerebrally into each of 10 mice weighting between 12-15gms in Virus Inactivation test. In NIH potency test, three fivefold dilutions each of reference and test vaccines were used for immunization of mice. A group of 16 mice were immunized with each dilution on day 0 and 7, followed by a challenge dose of 5-50 LD_{50} (Median Lethal dose) of Rabies Challenge Virus Standard (CVS) on day 14. The mice were observed for 14 days after challenge dose for the symptoms of fixed rabies virus. The ED_{50} (50 % effective dose) of reference and test vaccine were calculated by Reed and Muench formula and the relative potency is calculated through comparison with the reference vaccine. The relative potency of the all the four rabies vaccines were above the recommended potency of 2.5IU/single human dose. All the vaccines tested were found to be safe & potent irrespective of the strain of rabies virus, substrate and inactivation process used in production.

Keywords: Rabies, Cell Culture Rabies Vaccine, NIH Potency test, Virus Inactivation test.

1. Introduction

Rabies is acute fatal encephalitis that still takes heavy toll of human lives in many Asian, African and South American countries where dogs are the major vectors (>90%) of the disease. As per a WHO estimate nearly 55,000 people die of rabies globally every year [1, 2]. Rabies is endemic in India, where about 18,000 to 20,000 cases of rabies were reported and around 36% of the world's rabies deaths occur in India each year, most of those when children come into contact with infected dogs [3-5]. According to a study, only 70% of the people in India have ever heard of rabies, only 30% know to wash the wounds after animal bites and of those who got bitten, only 60% receive a modern cell culture derived vaccine [3, 6].

The production of rabies vaccine has dramatically changed since Pasteur, who produced and treated the patients with serial injections of increasingly virulent rabies virus infected nerve tissue by drying different time interval. In 1900s, Fermi and Semple used phenol to inactivate the rabies virus in nerve tissue. However, the use of the Fermi vaccines has been discontinued due to presence of residual live fixed rabies virus [7]. WHO has recommended that the production of all the nervous tissue vaccine should be discontinued and should be replaced by cell culture vaccines [8]. The first non-nervous tissue culture rabies vaccine became available in North America was purified Duck Embryo Vaccine (PDEC) which was replaced by first modern cell culture rabies vaccine, the Human Diploid Cell Strain Vaccine (HDCV) in USA in 1978 [9]. Further two cell culture vaccines namely Purified Vero Cell Culture Vaccine (PVRV) and Purified Chicken Embryo Cell Vaccine (PCECV) have been developed almost simultaneously and became available around the world by international pharmaceutical companies [10]. Clinical studies of modern cell culture rabies vaccines, such as HDCV, PCECV and PVRV for human use have established their reliable potency, immunogenicity, and

reasonable safety for both pre-exposure vaccination and post-exposure therapeutic application [11, 12].

The potency of inactivated rabies vaccine for animal & human use is tested by mouse protection test, which was originally developed at National Institute of Health (NIH), Bethesda, MD, USA. The NIH potency test was adopted by WHO expert committee on Rabies in 1950s and is part of many national & international requirements for evaluation of inactivated rabies vaccines [13]. The test measures the degree of protection conferred by inactivated rabies vaccines in immunized mice challenged with rabies virus [14]. As of other inactivated viral vaccines, avoiding the presence of residual virulent virus is of the utmost importance. For rabies vaccines the test for residual virulent virus is performed by intracerebral inoculation in mice [15].

The present study was carried out with a view to see the trend analysis of safety and potency of seventy batches of inactivated CCRV, produced by four indigenous manufacturers form India using different cell substrates, rabies fixed virus strains and inactivation methods.

2. Materials and Methods

2.1 Animals Used

Three to four weeks old *Swiss* mice weighing between 11-15g were used in this study. The experiments were approved by the Institutional Animal Ethical Committee (IAEC) of NIB as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). Compling to 3R principle for use animals, in order to reduce the severity of disease in test animals, mice were observed daily for the signs of rabies infection and whenever the signs indicated onset of rabies, mice were euthanatized humanly. Further to reduce the use of animals, two vaccine batches were tested at a time with a common reference vaccine and virus control. In a single performance of NIH assay, mice were grouped into 09 groups of 16 mice each, of which 03 groups of 16 mice were for reference vaccine and 06 groups for two test vaccine batches. Four groups of 10 mice each were kept as control groups for the titration of working Challenge virus standard (CVS). The groups were of same sex or in equal ratio of both sexes [16].

2.2 Vaccines and Virus

National Reference Standard (NRS) for rabies vaccine (9IU/vial), Rabies CVS-11 strain in mouse brain suspension and normal horse serum (NHS) were obtained from Central Research Institute (CRI), Kasauli, Himachal Pradesh, India. Inactivated cell culture rabies vaccines (n=70 batches) from four Indian manufacturers A, B, C & D were evaluated. Out of the four vaccine preparations, one was liquid adjuvanted vaccine, adsorbed to aluminum phosphate and three were lyophilized vaccines as mentioned in Table 1. The vaccines vary in rabies virus strain used for the production and the cell culture host system used for the propagation of virus. Three types of modern cell culture vaccines based on cell culture system i.e. PCECV, HDCV and PVRV were in evaluated in the present study.

Table 1: The details of the rabies virus strains and cell substrates used for production by the four rabies vaccine manufacturers

Manufacturer*	Rabies Virus Strain	Cell Culture system (Host System)	Batches Evaluated
A	Flury LEP	Purified Chick Embryo cell culture	31
B	Pitman-Moore (PM) strain	Purified Chick Embryo cell culture	20
C	Pitman-Moore (PM) strain	Human diploid cells culture	10
D	Pitman-Moore (PM) strain	Vero Cell Culture	09
Total			70

*Each manufacturer has been identified by an alphabet from A to D that is not related to this order of listing.

2.3 Virus Inactivation test by Mouse Inoculation:

Mouse inoculation test was performed to confirm virus inactivation using reconstituted lyophilized vaccine samples (0.03 ml), except for aluminum adsorbed liquid vaccine. The safety test for aluminum adsorbed liquid vaccine in mice is not done in this study. The mice were observed daily for 14 days for any rabies like symptoms (paralysis, convulsions) [15, 16].

2.4 NIH Potency test:

The three fivefold serial dilutions i.e. 1:125, 1:625, 1:3125 of the reference vaccine and the test vaccine were prepared in phosphate buffer saline (PBS) pH 7.6 [14]. Sixteen mice were immunized intraperitoneally with 0.5ml of each dilution of test vaccine or reference vaccine per mice using 26 gauge needles [16]. Each mouse received two doses of a particular vaccine on days 0 and day 7 by intraperitoneal route. The rabies CVS was diluted in 2% normal horse serum diluent (CVS diluent) to contain a challenge virus dose (CVD) of 5 to 50 LD_{50} in 0.03ml volume of single challenge [14]. All mice were challenged intracerebrally with a CVD of 5-50 LD_{50}/0.03ml of CVS on day 14 after the first dose of vaccine [16]. The three tenfold dilution i.e. 10^{-1}, 10^{-2} & 10^{-3} of CVS dilution containing CVD were made to conduct the titration of working CVS in four groups of 10 mice each were inoculated with 0.03ml with each CVS dilutions as a control. All mice were observed daily for 14 days after challenge and recorded the number of mice that died from rabies after the first five days. The mice showing signs of fixed-virus rabies on the 14th day were also included [14]. The death of mice within first five days of challenge dose were considered as

non-specific deaths not due to rabies virus infection. The ED_{50} of reference and test vaccine was calculated by Reed and Muench formula and the relative potency is calculated by comparing the effective dose of test vaccine with the reference vaccine [14, 17].

The geometric mean titers (GMT) and standard deviation (SD) of NIH potency results were calculated for each vaccine. The NIH potency results obtained were compared with the respective manufacturer's results. Analysis of variance was applied to calculate the difference in NIH potency results obtained by NIB with respective manufacturer.

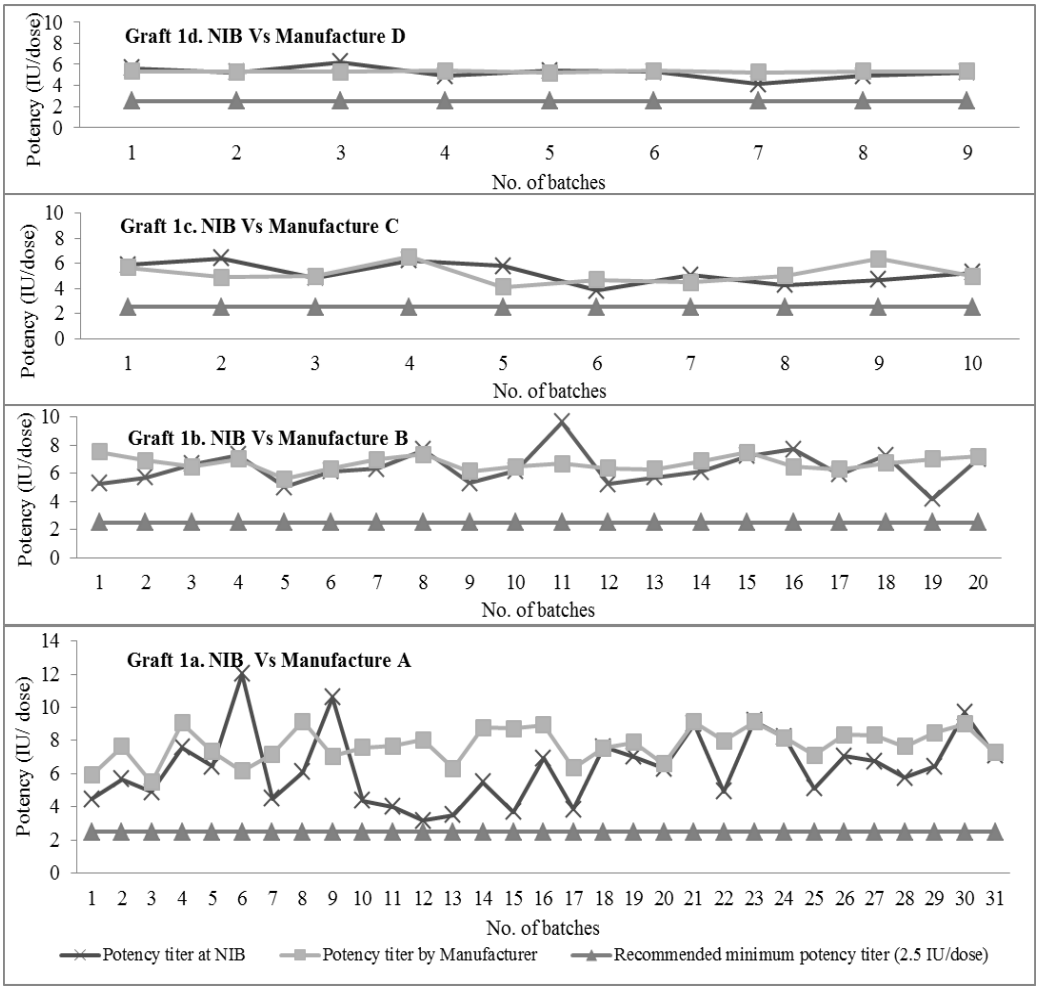
3. Results

The GMT of NIH potency results were found to be 5.64, 6.26, 5.16 and 5.18 for manufacturer A, B, C & D respectively. SD in NIH potency titers were 1.82, 1.22, 0.84 and 0.58 for manufacturer A, B, C & D respectively. The variance between the results of manufacturer A, B, C & D and NIB results were 2.94, 0.85, 0.59 and 0.15 respectively. The GMT of potency test results for the four vaccines used in this study are tabulated in Table 2. The trend analysis of potency titers of each vaccine obtained for manufacturer A, B, C, D as compared to NIB are graphically depicted in the Figure 1. All the rabies vaccines samples except for aluminum adsorbed-HDCV were found to be safe in virus inactivation test in mice.

Table 2: Geometric mean titers of different inactivated cell Culture rabies vaccines evaluated

Manufacturer	No. of batches tested	Results of NIB		Results of Manufacturer		Variance in GMT between manufacturer and NIB result
		GMT	SD	GMT	SD	
A	31	5.64	1.82	7.89	0.85	2.94
B	20	6.26	1.22	6.67	0.49	0.85
C	10	5.16	0.84	5.11	0.78	0.59
D	9	5.18	0.58	5.31	0.07	0.15

Figure 1: Trend analysis of potency titers estimated by NIB and manufacturers A, B, C, D.



4. Discussion

Following an order by the Supreme Court of India, the production and use of Semple rabies vaccine was discontinued since January 2005 and all dog bite victims attending both government and private clinics are now being administered with modern cell culture rabies vaccines. As a result, there is a huge demand for WHO approved modern cell culture rabies vaccines in India [18, 19].

The extensive quality control (QC) and independent testing of each lot of rabies vaccine by National Control Laboratory (NCL) is essential and mandatory as vaccine characteristics might vary for every batch produced under complex manufacturing procedures [20]. One of the major parameter of QC of inactivated rabies vaccine is potency which measures the product strength. Despite the multiplicity of seed viruses used in vaccine production, the PM strain used in reference vaccine preparations and the virus strain used for the challenge (CVS strain) in testing of rabies vaccine.

The cell culture rabies vaccines form Indian manufacturers, evaluated in the present study were found to be potent. However there are some variation in GMTs of potency test results between manufacturer and NIB, but all the vaccines were having titer above the WHO acceptable limit of 2.5 IU/single human dose for pre- and post-exposure prophylaxis [17]. Some of these variations are known to occur without affecting the results including the route of administration, number of doses, duration between vaccination and challenge, the age of the mice at the time of initial vaccination [21].

The results of the study suggest that vaccination with either of HDCV, PVCV or PCECV is safe and potent in mice irrespective of the strain of rabies virus, substrate and inactivation process used in production of rabies vaccines in India. However, quality evaluation for each lot of rabies vaccine is essential for the lot release by the respective National Control Laboratory.

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