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Research article

SAFETY AND IMMUNOGENICITY OF RABIES VACCINE SURE RAB™ (BIOMED) AND VERORAB™ (SANOFI PASTEUR).

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ABSTRACT: Rabies is the most dreaded zoonotic disease. Modern cell culture derived vaccines have been used for pre bite and post bite immunization. SURE RAB™ has been manufactured by BIOMED (P) Ltd. in Vero cells using Pitman Moore strain of Rabies virus. The clinical study was done to test the safety and immunogenicity of SURE RAB™ in pre and simulated post bite vaccination schedules for intramuscular (I/M) and intradermal (I/D) vaccination. VERO RAB manufactured by Aventis Pasteur was used for comparison. One hundred ninety four healthy volunteers who gave informed consents participated in the study at three centers: Meerut (U.P.), Aligarh (U.P.) and Bhavnagar (Gujarat). The volunteers were divided in seven groups who were vaccinated with either vaccine as per pre bite (I/M & I/D) or post bite (I/M & I/D – 2 Nos.) schedules. The reactogenicity was noted and immunogenicity was evaluated at 0, 7 &/or 14, 28-30 & 45 days of vaccination by ELISA test (BIO RAD) and RIFFIT tests. Both the vaccines were found to be minimally reactogenic and conferred equally good immune responses which far exceeded the WHO's requirements of ≥ 0.5 I.U. per ml. SURE RAB™ and VERO RAB proved equally safe and immunogenic when used as I/M or I/D schedules of vaccination. Statistically insignificant differences (95% C.I.) in immune response induced by three consecutive batches of SURE RAB™ validated the consistency of method of manufacture.

Keywords: Rabies, cell culture vaccine, pre and post bite regimens, safety & immunogenicity.

INTRODUCTION

Rabies is amongst the most dreaded zoonotic disease of human and animals. In India, dogs are the most important transmitter of disease as most of them roam as unowned, semi cared and unprotected against rabies among the human population both in Urban and Rural settings. Rabies has had an aura of tragedy as once clinical symptoms of disease develop in man or animal there was nothing that could protect the victim against sure death. The incidence of deaths due to rabies has been declining in India due to use of modern vaccines (A survey report, 2003). Availability of the first vaccine developed by Pasteur in 1885 led the world to know that rabies can be prevented if appropriate treatment is done immediately after exposure to animal bite followed by a series of vaccination to induce the body to respond and produce specific antibodies to halt the march of Rabies virus to brain tissue. The evolution of rabies vaccines over the last 125 years has probably resulted in successive improvements than any other vaccine against pathogenic microbial agent (Briggs *et al*., 2000). Modern cell culture derived, highly purified vaccines are in use all over the world for human (Belshe *et al*., 2004).

In contrast with most vaccines, rabies vaccination is resorted to on individuals already exposed to infection. Vaccination along with some clinical assistance, have resulted in nearly 100% saving of lives against rabies. The normal schedule after exposure consists of six vaccinations by intramuscular route on days 0, 3, 7, 14, 28 and 90 (optional). Antibodies appear 14 days onwards. In addition, two schedules of vaccinations – by intradermal route have been approved by the W.H.O. They have been adopted in Thailand, Philippines and Sri Lanka (WHO, 1984; Deshpande *et al*., 2003; Chhabra *et al*., 2005). The present clinical studies were conducted to evaluate the safety and immunogenicity of newly manufactured Rabies Vaccine, Human (Cell Culture) I.P. (SURE RAB^{TM}) → by BIOMED (P) LTD. as per various recommended schedules of vaccination. The study included both pre and simulated post exposure regimens (SPR) in healthy adults without history of rabies exposure or vaccinations. Multicenter trial at three medical centers

was done using three consecutive batches of SURE RAB^{TM} in India and one batch of one batch of VERORAB Rabies vaccine manufactured by Aventis Pasteur.

Materials and methods

Clearance and Approvals

The proposal to conduct the clinical trial was discussed independently by the ethics committees of three medical centers [LLRM Medical College, Meerut, U.P. (LLRM), Government Medical College, Bhavnagar, Gujarat (GMC), Jawaharlal Nehru Medical College, Aligarh, and U.P. (JNMC)]. All the centers cleared the protocols. No objection, for conducting the clinical trial, was given by the Drugs Controller (General) of India (D.C.G.I.) on February 13, 2009.

Vaccines

The vaccine was produced in vero cell culture using Pitman Moore strain of rabies virus. The virus was purified and inactivated with beta propiolactone. The vaccine contained > 2.5 I.U. of rabies antigen. Three consecutive batches were manufactured as per W.H.O. standards (W.H.O. T.R.S. No. 941, 2007) and I.P. as per GMP guidelines. They were tested by BIOMED and Central Drugs Laboratory, C.R.I., Kasauli, (H.P.) before use. Three batches were cleared on February 4, 2009. Batch No. R010808, R020808 and R030808 were used for trial at GMC, LLRM and JNMC respectively. The vaccine was in lyophilized form in single dose vial consisting of inactivated Rabies virus ≥ 2.5 I.U., Stabilizer – polygeline, sucrose and salts. The vaccine diluent was sterile water used for injection (1.0 ml).

The vaccine has been fully characterized in terms of physicochemical, biological properties and final manufacturing process. Consistency of manufacturing has been well documented. These lots are adequately representative of the formulation intended for marketing.

VERORAB^{TM} was manufactured by Aventis Pasteur using Rabies virus (Wistar strain PM/WI 38-1503-3M), in vero cell. The Rabies virus is inactivated by beta propiolactone. Vaccine is in lyophilized form in vials, containing inactivated rabies virus ≥ 2.5 I.U., stabilizer – maltose, human albumin, diluent 0.4% sodium chloride in distilled water. The batch number of

vaccine was B5572 bearing Mfg. Dt. 06/2007, Exp. Dt. 05/2010.

Subjects

One hundred ninety four volunteers participated in the study. All were healthy on clinical examination. They had not been previously vaccinated for rabies. None of the volunteers had history of sensitivity to any protein, common antibiotics, not addicted to alcohol or drugs, not pregnant, not taking steroids or immunosuppressive drugs and were mentally healthy and alert. Informed written consent was obtained from all volunteers prior to participation in the study. Majority of participants were in age group 19 years to 55 years, 5 were between 55-70 years age. Males were 86.6%, while females were 13.4%. None of the volunteers withdrew from the clinical trial for reason of side effects of the test/comparative vaccine.

Study Protocol

Open label, randomized controlled, multicentre, clinical trial. It was a two sided equivalence trial with outcome variables as antibody titer and the adverse events as measured by dizziness, fever, pain, headache, erythema, indurations and any other. The data of subjects were to be kept confidential. Volunteers at each center were randomly divided into seven groups. Group I & II were given `SURE RAB$^{TM}$, vaccine on day 0, 7, 28 by either intramuscular (I/M) or intradermal (I/D) route as per WHO schedule for pre-exposure vaccination. Group VI was given `VERORAB' as intramuscular injection as per pre exposure schedule. Group III and VII were given vaccine by I/M injection on day 0, 3, 7, 14 & 30 with `SURE RAB$^{TM}$, and `VERORAB' respectively as per simulated WHO schedule of post-exposure schedule of vaccination.

Group IV was given `SURE RAB^{TM}, as intradermal injection (0.1 ml) in upper deltoid region of both sides on 0, 3, 7 days and on one side on day 30 simulated post-exposure immunization as per Thai Red Cross Regimen (TRCR).

Group V was given `SURE RAB$^{TM}$, as simulated post-exposure immunization as per Oxford Regimen of intradermal injection of 0.1 ml each at eight sites on day `0' (on both sides of the deltoid, lateral thigh, suprascapular and lower abdominal region and lateral thigh and at one site in deltoid region on day 30.

Blood samples (1-2 ml) were drawn from Group I, II, III on day 0, 7, 14, 28, 45 and from group III, IV, V and VII on day 0, 7, 14, 30, 45. Serum was separated and stored in aliquots at or below -20^{o}C until required for testing or re-evaluation if required.

Volunteer Records

A detailed volunteer record file (VRF) was maintained which contained consent form, a detailed demographic profile including age, sex, address, medical history and general examination. Body temperature was assessed in each case and recorded if considered appropriate. Adverse reactions were noted as per protocol on every visit subsequent to vaccination.

Evaluation of Rabies Antibodies

Two methods were used to evaluate antibody response.

(i) ELISA Test:

Commercially available ELISA Test Kit (Platellia^{TM} - Rabies II kit manufactured by Bio Rad, U.S.A.) was used for titration of rabies antibody as per manufacturer's protocol. Positive controls were calibrated against the WHO standards (WHO, 1992). Negative and positive control sera were used with each test for validation, quality of detection and to establish the reference curves. All serum samples were tested in duplicate as per protocol. The reference curve was plotted with mean OD values. The curve was then used to calculate the titer of unknown sera samples.

(ii) Rapid Immuno Fluorescent Focus Inhibition Test (RIFFIT):

The protocol & test procedure used was the same as followed by the Center for Disease Control & Prevention, Atlanta, U.S.A (WHO, 1996). In these studies CVS-11 rabies fixed virus strain and mouse neuroblastoma cells and the standard Zeiss fluorescent microscope was used. The relative potency of test serum in international units (I.U.) per ml is determined by following formula

No. of I.U. /ml in test serum =
End point titer of test serum x 2 I.U. /
End point titer of Ref. serum diluted to contain 2 I.U. / ml

The rabies antibody titers of $\geq$ 0.5 I.U. / ml or more are considered protective (WHO, 1996).

CLINICAL EVALUATION PARAMETER

All volunteers were clinically examined to assess for safety parameters and the results duly recorded.

1. Local side effects (at the site of vaccination): Pain, erythema and inflammation were recorded 30 minutes, 4-6 hours and 48 hours after vaccination using scale of adverse reactions as (a) excellent – no local or systemic reactions. (b) Good – local reaction (inflammation) ≤ 5 cm in diameter and axillary temperature ≤ 38.3^{o}C (c) Fair- local reaction (inflammation) > 5 cm in diameter and axillary temperature ≤ 38.3^{o}C (d) Bad – local reaction (inflammation) > 5 cm in diameter and axillary temperature ≥ 38.3^{o}C.

2. Systemic side effects: Fever, diarrhoea, vomiting and any other side effects were recorded 30 minutes, 4-6 hours and 48 hours of vaccination.

Statistical Analysis

Quantitative variables were assessed for normality. In case of two groups, student test was used. For three groups, one way analysis of variance was done. Categorical variable were compared across groups using test of proportion or Fischer exact test. Summary along with 95% confidence interval were computed using STATA 10.0 statistical software.

Results

The analysis of data on age/sex distribution of volunteers in different groups represented an adequate mix up of various age groups.

None of the volunteers vaccinated either by intramuscular or intradermal method with `SURE RAB$^{TM}$, or `VERORAB' by any schedule of the vaccination, suffered from reactogenicity.

STATUS OF VOLUNTEERS WITH RESPECT TO RABIES ANTIBODIES

A.) **ELISA Test by Platelia^{TM} – Rabies-II Kit**

None of the volunteers had detectable antibodies (≥ 0.49 I.U.) in serum samples taken on day `0'. By day 14, all the groups had more than

0.5 I.U. in 100% cases. On day 28/30 and day 45, majority of volunteers had > 4 I.U. antibody titers in groups vaccinated as per `SPR' with either I/M and I/D groups. The details of the results are presented [Table 1].

Table 1: Pre and post vaccination serum samples titrated for rabies IgG antibodies by ELISA test – combined results of all three centers – (No. of volunteers %)

Day P/I	IU/ml	Gr. I	Gr. II	Gr. III	Gr. IV	Gr. V	Gr. VI	Gr. VII
		n = 27	n = 28	n = 28	n = 26	n = 28	n = 28	n = 29
Day 0	0.00 - 0.49	27 (100%)	28 (100%)	28 (100%)	26 (100%)	28 (100%)	28 (100%)	29 (100%)
	> 0.50	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Day 7	0.00 - 0.49	27 (100%)	28 (100%)	25 (89.3%)	25 (96.1%)	23 (82.1%)	28 (100%)	27 (93.1%)
	> 0.50	0 (0%)	0 (0%)	3 (10.7%)	1 (3.9%)	5 (17.9%)	0 (0%)	2 (6.9%)
Day 14	0.00 - 0.49	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	0.50 – 4.00	14 (52.0%)	20 (71.4%)	12 (42.8%)	12 (46.2%)	10 (35.7%)	16 (57.1%)	13 (44.8%)
	> 4	13 (48.0%)	8 (28.6%)	16 (57.2%)	14 (53.8%)	18 (64.3%)	12 (42.9%)	16 (55.2%)
Day 28/	0.00 - 0.49	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

	0.50 – 4.00	12 (44.4%)	20 (71.4%)	5 (17.8%)	6 (23.1%)	7 (25.0%)	13 (46.4%)	3 (10.3%)
	> 4	15 (55.6%)	8 (28.6%)	23 (82.2%)	20 (76.9%)	21 (75.0%)	15 (53.6%)	26 (89.7%)
Day45	0.00 - 0.49	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	0.50 – 4.00	6 (22.2%)	8 (28.6%)	2 (7.2%)	4 (15.4%)	6 (21.4%)	6 (21.4%)	3 (10.3%)
	> 4	21 (77.8%)	20 (71.4%)	26 (92.8%)	22 (84.6%)	22 (78.6%)	22 (78.6%)	26 (89.7%)

`SPR' = Simulated Post Exposure Regimen, Sure Rab$^{TM}$ was given to: Gr. I = Pre exposure I/M, Gr. II = Pre exposure I/D, Gr. III = `SPR' I/M, Gr. IV = `SPR' Thai Red Cross I/D and Gr. V = `SPR' Oxford I/D Sure Rab$^{TM.}$ VERORAB was given to: Gr. VI = Pre exposure I/M, Gr. VII = `SPR' I/M.

B.) **Rapid Immunofluorescent Focus Inhibition Test (RIFFIT)**

Serum samples taken on day 14, 28/30 and 45 were assessed in this test. The statistical analysis of results has been presented in Table 2 & 3. No significant differences in the sera response to either vaccine have been noted.

All volunteers were found to be immune (antibody titer of ≥ 0.5 I.U.) on day 14 and thereafter. The antibody titers were substantially higher when tested on 28/30 days. The titers further increased when tested 45 days after vaccination.

Table 2 : Statistical comparison (95% C.I) of pre and post vaccination serum titers of volunteers vaccinated with SURE RAB^{TM} or VERORAB using simulated post exposure intramuscular vaccination schedule : Antibody titrations were done by RIFFIT test – combined results of three centers

Day	Rabies Antibody Titers (RIFFIT) Group-III (SURE RAB^{TM})	Group-VII (VERORAB)	Wilcoxon rank sum t p-value
0	0.04 (0.03-0.05)	0.04 (0.03-0.05)	p = N.S. *
7	0.20 (0.15-0.28)	0.18 (0.12-0.26)	p = N.S. *
14	4.12 (3.04-5.58)	4.59 (3.58-5.88)	p = N.S. *
28/30	7.47 (5.43-10.26)	7.60 (5.99-9.63)	p = N.S. *
45	12.19 (8.77-16.94)	0.68 (8.30-13.74)	p = N.S. *

Table 3: Statistical comparison (95% C.I) of pre and post vaccination serum titers of volunteers vaccinated with SURE RAB^{TM} using various vaccination schedules. Antibody titration were done by RIFFIT test – combined results of three centers

Day	Rabies Antibody Titers (RIFFT)			p-value
	Group-III Intramuscular route (n = 28)	Group-IV Intradermal route `Thai-Red Cross' (n = 25)	Group-V Intradermal route `Oxford' (n = 28)	(Kruskal W Test)
0	0.04 (0.03-0.05)	0.05 (0.04-0.06)	0.04 (0.03-0.05)	p = N.S. *
7	0.20 (0.15-0.28)	0.22 (0.17-0.28)	0.35 (0.24-0.51)	p = N.S. *
14	4.12 (3.04-5.58)	4.42 (2.97-6.58)	4.43 (3.27-6.00)	p = N.S. *
28/30	7.47 (5.43-10.26)	7.72 (5.94-10.04)	6.69 (4.59-9.74)	p = N.S. *
45	12.19 (8.77-16.94)	11.34 (8.73-14.73)	9.42 (6.60-13.45)	p = N.S. *

* Group III Vs. Group V : $p<0.05$, Group IV Vs. Group V : $p<0.05$

Wilcoxon Rank Test with Corrected p-value for multiplecomparisons

Three batches of `SURE RAB™, vaccine used in the trial of various center induced similar antibody responses as tested by ELISA and RIFFIT tests.

Discussion

The SURE RAB™ manufactured by BIOMED (P) Ltd. and VERORAB™ manufactured by Aventis Pasteur were well tolerated. All cases of pain, erythema and headache were considered to be of low severity and resolved without the need of any interventions. No significant differences were found between the two vaccines when compared on the basis of adverse events, either local or systemic.

Immune response to rabies vaccines in the form of neutralizing antibodies have been reported to correlate with the protection against rabies. Test for immune response by Elisa and RIFFIT methods yielded identical results with Sure Rab™ and VERORAB vaccines. Similar observations have been reported (WHO, 1992; Chhabra *et al*., 2005; Mahendra *et al*., 2009).

All of the volunteers vaccinated as per pre bite schedule either by I/M or I/D route had antibody titers of ≥ 0.5 I.U (WHO, 1997).

In the post bite schedule, no significant differences in antibody response (95% C.I.) could be noted in groups vaccinated with either vaccine by intradermal route (Oxford or Thai Red Cross Regime) or by intramuscular route (0, 3, 7, 14, 28/30 group). The serum antibody titers were ≥ 0.5 I.U. in all volunteers when tested on day 14 after vaccination. The antibody titers increased with the time as reflected in titers obtained on samples taken on day 28/30 and 45. The multiple vaccination schedules induced the production of antibodies rapidly and attained substantially higher titers in all groups vaccinated by Intramuscular or

intradermal schedules. Similar results have been noted by other workers (Perrin *et al*., 1980; Chhabra *et al*., 2005; Mahendra *et al*., 2009).

Three consecutive batches of SURE RAB^{TM} – one batch per study center, induced statistically indistinguishable antibody titers (95% C.I.) in volunteers of all groups establishes consistency of process of manufacture of the vaccine.

Thus intradermal route proved as good as intramuscular route as it induces higher levels of immunity while using much smaller doses of the vaccine. Similar observations have been reported for Hepatitis B (WHO, 2007) and influenza vaccine (Redfield *et al*., 1985). The Oxford regimen of post exposure regimen involving I/D vaccination of 0.1 ml at each of 8 sites on day `0' appears to be the best option. This method requires least number of visits for vaccination.

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