Title: WHETHER HIGHER ANTIGENCITY PRODUCES HIGHER IMMUNOGENICITY IN INTRADERMAL RABIES VACCINATION? RESULTS OF A METAANALYSIS

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Keywords Rabies vaccines, Intradermal route, antigenicity, immunogenicity, metaanalysis

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Original Article

Whether higher antigenicity produces higher immunogenicity in intradermal rabies vaccination? Results of a metaanalysis

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Abstract

The metadata of 10 published studies and 3 vaccine trial reports comprising of 19 vaccine cohorts from four countries conducted over a period of 23 years (1986 - 2009) was used for metaanalysis. The vaccines studied were purified chick embryo cell vaccine (Rabipur, India & Germany), purified vero cell rabies vaccine (Verorab, France; Indirab, India) & human diploid cell vaccine (MIRV, France). The potency of these vaccines varied from 0.55 IU to 2.32 IU per intradermal dose of 0.1ml per site. The vaccines were administered to 1011 subjects comprising of 19 cohorts and using five different ID regimens. The immunogenicity was measured by assays of rabies virus neutralizing antibody (RVNA) titres using rapid fluorescent focus inhibition test (RFFIT) [15 cohorts] and mouse neutralization test (MNT) [4 cohorts]. The statistical analysis of the data was done by Mann-Whitney test. The results showed that a higher antigenicity did not produce a significantly higher immunogenicity in intradermal rabies vaccination ($p > 0.331$ & $p > 0.482$).

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Introduction

The intradermal rabies vaccination (IDRV) using selected cell culture vaccines(CCVs) has been established as an efficacious and economic alternative to the standard intramuscular (IM) regimens. IDRV has been successfully operational for post-exposure prophylaxis (PEP) in developing countries such as Thailand, Philippines, Srilanka and recently introduced in India in 2006.

Currently, three types of cell culture vaccines are available for application by intradermal (ID) route for prevention of human rabies. These are human diploid cell vaccine (HDCV), purified chick embryo cell vaccine (PCECV) and purified vero cell rabies vaccine (PVRV). As per the recommendations of World Health Organization (WHO), these vaccines shall have an antigen content as measured by potency of at least 2.5 international units (IU) per IM dose. This potency is expected to produce a rabies virus neutralizing antibody (RVNA) response of = 0.5 IU per ml in the vaccinees, which is considered as adequate for protection against rabies and this holds good for ID regimens too. However, WHO has neither prescribed an upper limit of potency for CCVs by intramuscular route nor recommended the potency per ID dose. Whereas, the national regulatory authorities for procurement of rabies vaccines for ID route in Thailand and Srilanka advocate a minimum potency of 0.7 IU in 0.1 mL per ID site[1] and in Philippines 0.5 IU in 0.1 mL per ID site.

The dose of HDCV and PCECV is 1 mL by IM route, whereas the dose of PVRV is 0.5 mL. However, the ID dose of all the three vaccines is 0.1 mL per ID site, irrespective of the volume by IM route. Besides, the use of different ID regimens viz. 8 site, 4 site, 2 site, etc. result in different antigenic loads i.e., the total amount (in IU) of antigen injected in the vaccinees, as the volume of vaccine administered varies according to the number of sites injected.

In this background, having conducted previously a metaanalysis assessing the relationship between antigenicity and immunogenicity of human rabies vaccines by IM[2] and ID[3] routes the authors undertook this extended appraisal to know whether an higher antigenicity produces higher immunogenicity in IDRV.

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Materials and Methods

The authors used Pubmed and selected ten studies published in peer reviewed national/international journals[4-13] and that were conducted to evaluate the immunogenicity of rabies vaccines by ID route. Besides, the clinical trial reports of three Indian studies (not yet published) were also included[14-16]. The metadata of these thirteen studies from four countries i.e., India, Thailand, Germany & Lithuania conducted over a period of 23 years (1986-2009) was used for metaanalysis.

Table 1
Details of regimens, schedules, cohorts and vaccines

Regimen	Schedule	Total cohorts	Vaccines (cohorts)
Updated TRC	(2-2-2-0-2)	5	Rabipur (2) Verorab (1) Indirab (2)
TRC	(2-2-2-0-1-1)	7	Rabipur (4) Verorab (3)
8 site	(8-0-4-0-1-1)	2	Rabipur (2)
4 site	(4-0-2-0-1-1 & 4-4-4-0-1-1)	4	Rabipur (2) Verorab (1) MIRV (1)
KIMS	(2-2-2-2-2)	1	Rabipur (1)

Note: RVNA Method: RFFIT (15 cohorts); MNT (4 cohorts)

The total number of subjects in these thirteen studies having nineteen vaccine cohorts was 1011. The vaccines studied were PCECV (Rabipur, India and Germany), PVRV (Verorab, France and Indirab, India) and HDCV (Merieux inactivated rabies vaccine, France). The different ID regimens used were- updated TRC [2-2-2-0-2] 5 cohorts; TRC [2-2-2-0-1-1] 7 cohorts; 8 site [8-0-4-0-1-1] 2 cohorts; 4 site [4-0-2-0-1 & 4-4-4-0-1-1] 4 cohorts and KIMS [2-2-2-2-2] 1 cohort. The RVNA assessment was done by using rapid fluorescent focus inhibition test (RFFIT) in 15 cohorts and mouse neutralization test (MNT) in 4 cohorts (Table 1). Both the methods are approved by WHO.[17] The data available from nineteen cohorts included potency of the vaccine, ID schedule, number of subjects and geometric mean concentrations of RVNA for days 14 & 90. The potency of rabies vaccine per 0.1 mL of ID dose was calculated depending on the volume and potency of the same vaccine used by IM route. The "antigenic load" is defined as the amount of antigen injected by days 7 & 28 according to the potency of vaccine & ID regimen used. The antigenic load was computed for all the nineteen vaccine cohorts from thirteen studies. The GMCs of RVNA assays were noted for the days 14 & 90 as a measure to assess the immune response to the antigenic load/stimulus by days 7 & 28 respectively (Table 2).

Table 2 Details of vaccine cohorts, ID regimens, antigenic loads and immune response

Cohort	Vaccine	Potency /ID dose	ID Schedule	Number of Subject	Antigenic load (Day 7)	GMC (Day 14)	Antigenic load (Day 28)	GMC (Day 14)	Type of RVNA analysis
1.	Rabipur[4]	0.55	4-0-2-0-1-1	86	3.30	20.50	3.85	2.39	RFFIT
2.	Indirab[16]	0.55	2-2-2-0-2	55	3.30	5.40	4.40	3.66	RFFIT
3.	Rabipur[5]	0.60	2-2-2-0-1-1	65	3.60	7.13	4.20	2.27	MNT
4.	MIRV[6]	0.32	4-4-4-0-1-1	19	3.84	9.88	4.16	3.57	RFFIT
5.	Rabipur[7]	0.32	4-4-4-0-1-1	19	3.84	9.88	4.16	3.57	RFFIT
6.	Rabipur[8]	0.76	2-2-2-0-1-1	25	4.56	6.70	5.32	5.10	MNT
7.	Rabipur[16]	0.91	2-2-2-2-2	54	5.46	4.75	7.28	3.30	RFFIT
8.	Rabipur[9]	0.92	2-2-2-0-1-1	59	5.52	28.50	6.44	3.00	RFFIT
9.	Rabipur[10]	0.94	2-2-2-0-2	45	5.64	4.17	9.43	4.79	RFFIT
10.	Rabipur[11]	0.94	2-2-2-0-1-1	49	5.64	4.30	6.58	6.70	RFFIT
11.	Rabipur[14]	0.99	2-2-2-0-2	81	5.94	5.83	7.92	7.20	RFFIT
12.	Rabipur[12]	0.75	4-0-4-0-1-1	15	6.00	7.20	6.75	2.70	RFFIT
13.	Indirab[15]	1.14	2-2-2-0-2	68	6.84	4.46	9.12	4.30	RFFIT
14.	Rabipur[12]	0.75	8-0-4-0-1-1	15	9.00	5.40	9.75	2.70	RFFIT
15.	Rabipur[13]	0.78	8-0-4-0-1-1	39	9.36	10.20	10.14	8.50	MNT
16.	Rabipur[13]	1.56	2-2-2-0-1-1	43	9.36	6.80	10.92	5.80	MNT
17.	Verorab[4]	1.78	4-0-2-0-1-1	87	10.68	26.10	12.46	2.75	RFFIT
18.	Verorab[13]	2.24	2-2-2-0-2	66	13.44	4.67	17.92	4.75	RFFIT
19.	Verorab[9]	2.32	2-2-2-0-1-1	59	13.92	28.90	16.24	2.70	RFFIT

Note: (i) GMC = Geometric mean concentration.
(ii) RFFIT = Rapid fluorescent focus inhibition test.
(iii) MNT = Mouse neutralization test.

In the absence of a WHO recommendation of potency for CCVs by IDRV, the norm advocated by Governments of Thailand and Srilanka for procurement of vaccines for IDRV viz. 0.7 IU per 0.1 ml of ID dose was used for appraisal in this study. Considering the standard ID regimen of updated TRC (2-2-2-0-2), the quantum of antigen injected ie., antigenic load by day 7 (viz.0.7 IU X 6 doses = 4.2 IU) and day 28 (viz.0.7 IU X 8 doses = 5.6 IU) was used to bifurcate the vaccine cohorts into two categories.viz., those with lower antigenicity i.e., = 4.2 IU by ID route by day 7 and = 5.6 IU by ID route by day 28; those with higher antigenicity i.e., > 4.2 IU by ID route by day 7 and > 5.6 IU by ID route by day 28. The corresponding combined geometric mean concentrations (cGMCs) of RVNA by day 14 (for antigenic load by day 7) and of day 90 (for antigenic load by day 28) were calculated as criteria to measure the immunogenicity response to the antigenic loads.

Table 3

Evaluation of antigenicity versus immunogenicity: Combined geometric mean concentrations of rabies virus neutralizing antibody response to different antigenic loads on different days – Results of Mann-Whitney test.

Antigenic load	Sample size (Subjects)	Combined geometric mean concentrations (cGMCs)	Z - value	P - value
By day 7		**By day 14**		
• Low (< 4.2IU)	306	9.93 IU	0. 973	> 0.331
• High (> 4.2 IU)	705	8.65 IU		
By Day 28		**By day 90**		
• Low (< 5.6 IU)	331	3.06 IU	0.703	> 0.482
• High (> 5.6 IU)	680	4.10 IU		

Results

To evaluate the differences in cGMCs in the two antigenicity groups, the Mann-Whitney test was used. The results revealed paradoxically that an higher antigenicity / antigenic load by day 7 produced a lower immune response (cGMC) by day 14. On the contrary, an higher antigenicity / antigenic load by day 28 produced a higher immune response (cGMC) by day 90. However, in both instances the results were found to be statistically not significant (Table 3).

Discussion

Rabies being a practically 100% fatal disease, IDRV is a life saving treatment in PEP which involves administration of small quantity i.e. 0.1 mL of vaccine into the dermis layer of skin. As rabies is endemic in the developing world, IDRV is recommended as a cost effective tool for use in countries of Asia and Africa. Some consider IDRV as an "inferior/weaker cousin" of intramuscular rabies vaccination, throwing doubt on its efficacy. Compounding this problem further, WHO having defined potency for rabies vaccines by IM route, has not done so, for rabies vaccines given by ID route. Consequently, some countries like Thailand, Srilanka and Philippines have mandated a higher potency for IDRV.

In a study using a single lot of PCECV vaccine with potencies ranging from 0.032 IU (1:16 dilution) to 0.506 IU (undiluted) per ID dose of 0.1 mL when administered to subjects showed a clear, almost linear relationship discernible between the amount of antigen administered and immune response in terms of RVNA titres.[18] A study involving Vietnamese children which used three batches of PVRV with potencies ranging from 3.5 IU to 12.0 IU per vial (of 0.5mL) failed to show a definite dose-response relationship.[19] Another study using dilutions of HDCV when administered intradermally to healthy volunteers demonstrated a dose-response relationship.[20]

Following a metaanalysis, the authors found that there was no significant linear relationship between antigenicity and immunogenicity of rabies vaccines when administered by ID routes.[3] As an extension of it, the authors following this further appraisal observed that a higher antigenicity did not produce significantly higher immunogenicity in IDRV.

This reiterates that the current WHO recommendation of a minimum potency of 2.5 IU per IM dose i.e., 0.5 mL for PVRV and 1 mL for HDCV and PCECV, which amounts to 0.5 IU per ID dose of 0.1 mL for PVRV and 0.25 IU per ID dose of 0.1 mL for HDCV and PCECV is adequate for ensuring an efficacious IDRV.

As this is an explorative study, the authors recommend that in future the meta analysis may be done by considering more number of studies as and when papers on IDRV are published, giving a greater power for analysis. For the present, the findings of this study hopefully should convince the authorities, medical profession and the industry to have confidence in the potencies of rabies vaccines currently advocated for use by intra dermal route.

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ANNOUNCEMENT

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