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EVALUATION OF SAFETY AND IMMUNOGENICITY OF ABHAYRAB™ ID REGIMEN ON HEALTHY VOLUNTEER

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Background: Rabies is a fatal, acute, progressive viral encephalitis which is only preventable by vaccine. However, multiple times injection schedule and high cost have limited the usage of the rabies vaccine. Compared to IM regimen, ID regimen has advantage of lower cost, unfortunately, it is not used widely because of difficult injection technique, vaccine wasted, cost-benefit of provider and limitation of safety and immunogenicity profile, especially in Vietnamese population. We aimed to assess the safety and immunogenicity of Abhayrab™ ID regimen. The results might encourage the widely application of ID regimen.

Methods: We undertook an open-labeled in one group phase 4 study between Oct 1, 2013 and Apr 30, 2014 at Long Xuyen city, An Giang province, Vietnam. The participants (aged 18 – 66) were administered intradermally two doses of vaccine at deltoid regions of two arms at the day 0, 3, 7 and 28. Three blood samples were collected before vaccination at the day 28 before two last doses administration and the day 180. Solicited adverse events and unsolicited adverse events would be followed for 3 days and 30 days post-vaccination whereas serious adverse events were assessed during the trial.

Results: Immunogenicity based on per-protocol population showed that GMT values on 28th and 180th day are 2,618 IU/mL (95% confidential interval, 2,441 – 2,795) and 0,695 IU/mL (95% confidential interval, 0,466-0,924), respectively. For safety, the most common solicited local and systemic AEs are itching (17%), redness (10%) and headache (13%), fatigue (12%), body itching (9%), dizziness (6%) respectively. Most of these AEs were mild and occurred within first three days post-vaccination. No unsolicited AEs and SAEs were reported.

Conclusions: Abhayrab™ vaccine ID regimen is well tolerated, safe and immunogenic on healthy Vietnamese, widely application of this regimen will give more opportunities for people to be administered post-exposure, therefore reduce rabies-induced deaths.

Introduction

Rabies was transmitted from domestic and wild mammals, particularly dogs and related canid species, raccoon, mongooses, skunks and bats to human through bites. There are two forms of rabies in human: furious and paralytic. Until recently, there had still been no successful therapy of rabies [1]. The annual number of human rabies deaths estimated in 2010 is from 26400 (95% CI: 15200 to 45200) to 61000 (% CI: 37000 to 86000), the vast majority of deaths occurs in the rural area (84%) in developing countries in Asia, Africa and Latin America [2]. All survivors had received pre-exposure or post-exposure vaccination. Before 1996, Vietnam had 350,000 – 450,000 people exposure to rabies virus and approximately 500 deaths each year because they had not received full schedule of vaccine and immunoglobulin on time [3]. AbhayrabTM was marketed in Vietnam since 2009 with two regimens IM and ID. However, the ID regimen was rarely used due to lack of clinical data about efficacy and safety as well as difficulties of ID injection skill in spite of its low cost. Therefore, we conducted this trial to provide immunogenic and safety data on Vietnamese, which then encourages widely application of the ID regimen.

Methods

Study design and participants

We undertook an open-labeled, phase 4 study, compared immunogenicity pre- and post-vaccination in the same group of participants in Long Xuyen city, An Giang province where there is high ratio of rabies vaccine ID regimen injection since last two year (2012-2013).

We enrolled 100 healthy men and women who satisfied following selection criteria: 1) aged from 18 years; 2) women participants had to have negative an urine pregnancy test at screening day, for females of childbearing potential who are sexually active agreed to use an acceptable contraceptive method during the study; 3) receipt of no any previous rabies vaccine; 4) understanding and compliance with trial procedure; 5) signing and dating a written informed consent prior to the initiation of any trial procedures after the nature of the trial had been explained. Participants ineligible if they 1) were using IV administration immunoglobulin or immunosuppressive drugs such as systemic corticosteroids, anticancer drugs, chloroquine...2) had autoimmune diseases or immunodeficiency at screening day; 3) known allergy to any components of investigational vaccine; 4) were pregnant or breastfeeding; 5) intellectual deficiency. In total, we enrolled 100 subjects aged from 18 year (except for 1 subject who missed 14 days to reach 18 year olds at enrollment time).

The clinical trial protocol was approved by the Ethics Committee of Vietnam Ministry of Health and in accordance with the principles of the Declaration of Helsinki, Good Clinical Practices.

Outcomes

Our primary endpoints were evaluation of safety and immunogenicity of AbhayrabTM ID regimen on healthy Vietnamese adults. In detail, we assessed immunogenicity of Abhayrab including GMT, ratio of seroconversion participants on day 28 before two last doses and on day 180. For safety,

we analyze incidence and ratio of solicited AEs within 3 days post vaccination, unsolicited AEs within 30 days post-vaccination after each vaccination, and SAEs during study.

Procedure

AbhayrabTM was manufactured from vero cells by Human Biologicals Institute. Vaccine was transferred and stored at 4 °C. If subjects agreed to participate into trial after being explained by investigator, they would be physical examination to check criteria for inclusion and exclusion. Enrolled participants would receive two doses of AbhayrabTM by intradermal administration on day 0, 3, 7 and 28. However, before vaccination on day 0, day 28 and on day 180, participants were taken 3 mL of blood sample to check antibody response. Solicited adverse events and unsolicited AEs were recorded in diary cards by participants for three days and 30 days, respectively after each vaccination. The diary cards were collected in next visit except the 4th diary card which is collected on day 30 of last dose on day 28 to avoid the risk of diary card lost due to long interval between the last vaccination and the last visit for blood collection at 6 month visit. Solicited systemic adverse events assessed including fever, headache, myalgia, fatigue, dizziness, joint pain. Solicited local adverse events monitored including pain, redness, swelling/induration, itching at injection site. We documented and followed up serious adverse events that happened any time during the study.

We measured titres of anti-rabies virus neutralizing antibody in sera collected on day 0, 28 and 180 after first immunization

by rapid fluorescent focus inhibition test (RFFIT).

Statistical analysis

We summarized antibody responses with geometric mean titres and ratio of seroconversion population at 95% CI. Participants having antibody titres above 0.1 IU/mL at day 0 will be considered as rabies vaccination in the past and not be incorporated into immunogenicity-analyzed population. On day 28 and 180, AbhayrabTM ID regimen shows protective effect against rabies if antibody titres ≥ 0.5 IU/mL and by contrast.

We summarized AEs with ratio. Statistical analyses were by intention to treat for safety and per protocol for immunogenicity.

Role of funding source

The study was sponsored by Center of public health research and consultation (CRCCH), AbhayrabTM vaccine was provided by Duc Minh Medical JSC/ Indian Immunological Limited.

Results

We invited 236 subjects to provide general information about study. 117/236 (49,6%) subjects came back to site on screening day to be explained more detail about the nature of study. There were 116 subjects signed informed consent form and to be physical examination. The remaining one subject previously injected with rabies vaccine so did not sign ICF. However, there were only 100 participants came and were vaccinated on the first vaccination day. 60% of the study population was women (table 1). The median age of participants was 35 years (range 17-66), one participant's age was 17 year 9 month when enrolled into study due to document monitoring mistake. 13

participants confirmed to be previously bitten by animals but did not receive rabies vaccination. On day 3, four participants withdrawn from study because of 1) Moving to another place for working; 2) difficulty in time arrangement; 3) based on advices of relative who works in medical field; 4) appearance of itching within hand. Finally one participant was not collected blood sample on day 180 due to moving another place.

There were no serious adverse events reported during study. One participant who was bitten by dog three months after the last vaccination on day 28 was not indicated with rabies vaccine. Most of solicited adverse events were mild or moderate (Supp. 1). After day 0 post-vaccination, the ratio of systemic adverse events was equal to that of local adverse events, and gradually decreased in next injections (table 2). Headache and fatigue were the most commonly reported systemic adverse events. 13% and 12% participants had headache and fatigue, respectively after the first vaccination, however, these ratio declined dramatically after vaccination on day 7 and day 28 with 5% of headache, 3% of fatigue on after day 7 and 2% of headache, 2% of fatigue after day 28, respectively (table 3). For solicited local adverse events, itching and redness were the most commonly reported with 17% and 11% participants appeared these AEs after first vaccination and slightly changed after second and third vaccinations. These adverse events significantly reduced after fourth vaccination (table 4).

Immunogenic analysis based on per protocol population including 80 participants. 20

participants were excluded because of protocol deviation: 1) 14 participants had anti-rabies antibody titres ≥ 0.1 IU/mL pre-vaccination; 2) 3 participants did not complete the injection schedule; 3) 1 participant did not comply injection schedule as defined in the protocol; 4) 1 participant aged 17 years at enrolled time; 5) 1 participant was not collected blood sample on day 180. Before two last doses on day 28, 100% per protocol population reached antibody titres above 0.5 IU/mL, in which 89% population reached over 5 IU/mL. The antibody response gradually decreased in a time-dependent manner. Six months later since first vaccination, 94% participants still maintained protective antibody threshold with 15% participants showed antibody titres above 5 IU/mL (table 5). The seroconversion ratio reached 100% from non-protective to protective antibody level on day 28. 6.25% participants had seroconversion from protective (on day 28) to non-protective antibody level on day 180. Among 5 participants who had under-protective antibody level on day 180. We did not correlate antibody level decrease of these participants with baseline as well as geographic characteristics (data not shown). GMT values on day 28 (after six doses of AbhayrabTM intradermal administration) and on day 180 (5 months since last doses) were 2.618 (95% CI: 2.441-2.795) and 0.695 (95% CI: 0.466-0.924), respectively. GMT_{180} declined significantly compared to GMT_{28} although it was still higher than protective threshold (table 6). We performed extra analysis for 14 participants who had antibody titres above 0.1 IU/mL, it is ranged from 0.11 to 2.0 IU/mL, 10 subjects had

under-protective antibody level (0.5 IU/mL) and 4 subjects had upper-protective antibody level. This results proved that these subjects had administered rabies vaccines before entering the studies but they did not remember or forget to declare in the screening day. GMT₂₈ and GMT₁₈₀ in this group were 3.858 (95% CI: 3.287-4.413) and 2.198 (95% CI: 1.604-2.792), respectively and were significantly higher than previously unvaccinated group (table 7).

Discussion

At the time of vaccine administration in the study, 86% participants did not have anti-rabies antibody and 14% participants had anti-rabies antibody, it means that these participants had previously administered rabies vaccine.

Antibody concentration increased considerably at day 28, GMT₂₈ reached 2.618 IU/mL (95% CI: 2,441-2,795) – five times higher than protective threshold antibody titres. However this level is still lower than GMT₂₈ in a study conducted in Philippin, in which GMT₂₈ is 4.82 IU/mL (95% CI: 3.90-5.97) [4]. We suspected the difference between two studies about GMT₂₈ is attributable to investigational population's age. The study in Philippine enrolled subjects aged from 5 to 50 whereas participants in our study aged from 17 to 60 years.

Our study also observed the substantial decline of antibody level compared to that of day 28 after 6 months since the first doses. Certainly, 15% participants remained the antibody concentration level over 5 IU/mL, GMT₁₈₀ declined down to 0.695 IU/mL (95% CI: 0.466-0.924). This result supports

for the administration of booster doses. According to recommendation of WHO for ID regimen, if subjects are pre-exposure administrated with rabies vaccine, they will be boosted 2 doses when exposure.

In a study conducted in India, GMT₂₈ and GMT₁₈₀ were 11,04 (CI 95%: 7,24 – 16,83) and 3,31 (CI 95%: 2,01 – 5,45), respectively which was much higher than results observed from our study [5]. It was possible that small sample size and a booster dose on day 90 in the study in India were attributable for this difference. However, the decline of antibody level from day 28 to day 180 was not significantly different between two studies. Although GMT₁₈₀ in our study was lower than 2 other studies as described above but it was still higher than level indicated for ID regimen (approximately 0,5 IU/mL) and much lower than level of IM regimen (above 1 IU/mL) in a review study [1].

Itching (17%) and redness (10%) were most commonly local AEs but they were lower than WHO's report in which 35-40% subjects appeared redness, pain, swelling at injection site [6]. Redness and itching occurred at lower frequency, in contrast, pain occurred at higher frequency in comparison with Philippin's study (table 8). Fatigue and headache, appeared from 12 - 13 %, were the most commonly systemic AEs after the first doses whereas fever occurred around 5% participants. These ratios located within WHO reported range for ID regimen rabies vaccine which was 5-15%.

Conclusions

Our study showed that Abhayrab™ ID regimen was well tolerated, safe and

induced antibody response which should be applied more commonly in Vietnam and then reduced rabies-induced death ratio.

References

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Figure 1: Trial profile

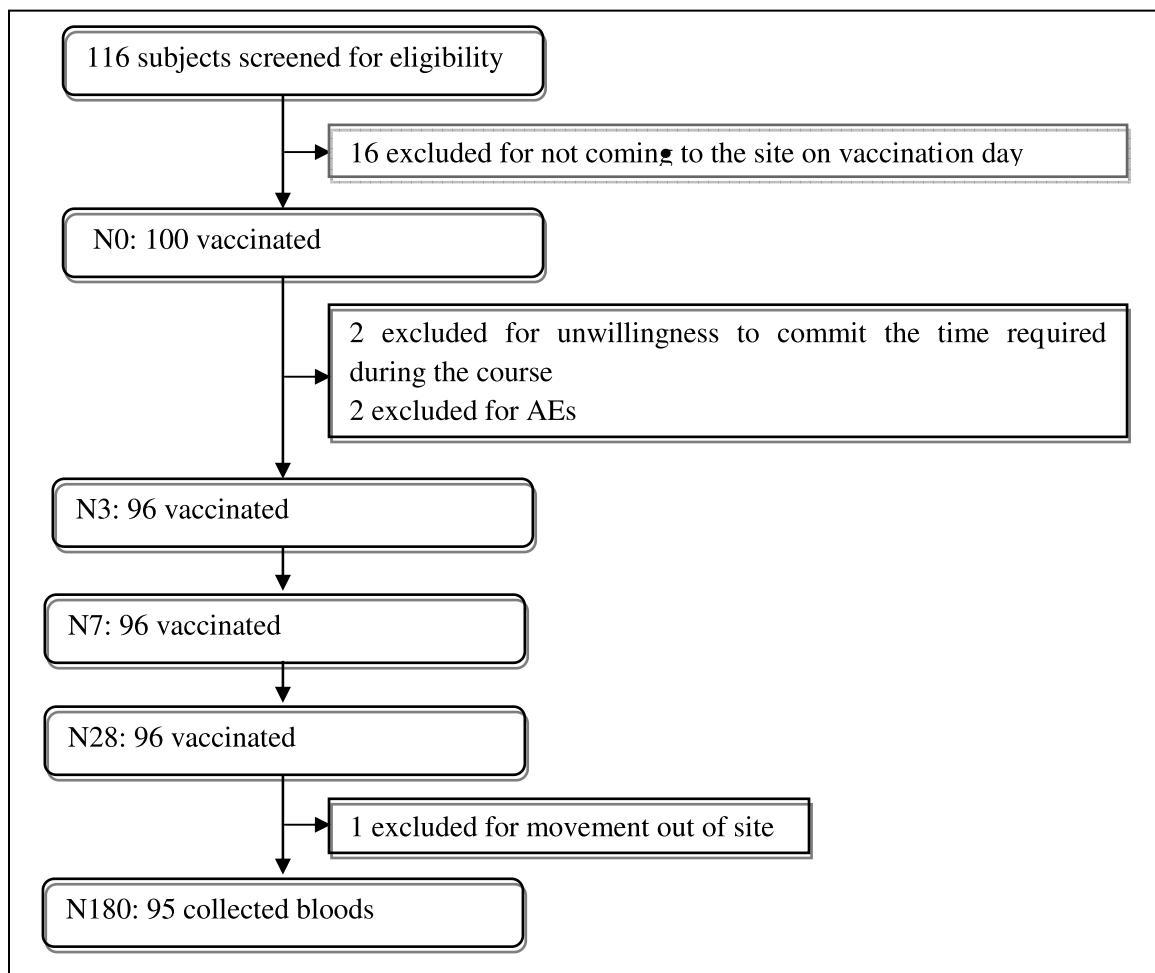


Table 1: Baseline characteristics

Sex	
Male	40 (40%)
Female	60 (60%)
Age at screening (years)	35 (17-66)
Weight (kg)	53 (32-91)
Data are n (%) or median (range), unless otherwise indicated.	

Table 2: Percentage of patients who had at least one AEs after each injection

≥ 1 AEs	After N0 (n,%)	After N3 (n,%)	After N7 (n,%)	After N28 (n,%)
Local	25 (25%)	26 (27%)	17 (18%)	11 (11,5%)
Systemic	27 (27%)	14 (14,5%)	9 (9,3%)	7 (7,3%)

Table 3: Percentage solicited local AEs after each injection

Solicited local AEs	After N0 (n,%)	After N3 (n,%)	After N7 (n,%)	After N28 (n,%)
Redness	10 (10%)	11 (11,5%)	11 (11,5%)	6 (6,3%)
Swelling	3 (3%)	1 (1%)	0	3 (3,1%)
Pain	6 (6%)	7 (7,3%)	6 (6,3%)	3 (3,1%)
Itching	17 (17%)	17 (17,7%)	10 (10,5%)	4 (4,2%)

Table 4: Percentage solicited systemic AEs after each injection

Solicited systemic AEs	After N0 (n,%)	After N3 (n,%)	After N7 (n,%)	After N28 (n,%)
Fever	5 (5%)	0	0	0
Erythema	9 (9%)	1 (1,1%)	0	4 (4,2%)
Fatigue	12 (12%)	10 (10,4%)	3 (3,1%)	2 (2,1%)
Headache	13 (13%)	9 (9,4%)	5 (5,2%)	2 (2,1%)
Dizziness	6 (6%)	2 (2,1%)	0	1 (1%)
Joint-pain	2 (2%)	3 (3,1%)	2 (2%)	0
Myalgia	6 (6%)	3 (3,1%)	3 (3,1%)	3 (3,1%)

Table 5: Comparison of antibody level before vaccination, before two last doses and after 6 months since the first doses

Antibody concentration	N0 n (%)	N28	N180
		n (%)	n (%)
No (< 0,1 IU/mL)	80 (100%)	0	0
Non-protective (< 0,5 IU/mL)	0	0	5 (6%)
Protective			
Từ 0,5 đến 5 IU/mL	0	9 (11%)	63 (79%)
Trên 5 đến 10 IU/mL	0	12 (15%)	5 (6%)
Trên 10 đến 20 IU/mL	0	42 (52%)	4 (5%)
Trên 20 đến 30 IU/mL	0	6 (8%)	0
> 30 IU/mL	0	11 (14%)	3 (4%)
Tổng số	80	80	80

Table 6: GMT28 and GMT180 of unvaccinated group (per protocol analysis)

Day	n	GMT	CI 95%	SD	Min, max
N28	80	2,618	2,441-2,795	0,088	1,081;4,434
N180	80	0,695	0,466-0,924	0,114	-0,916;3,964

Table 7: GMT28 and GMT180 of vaccinated group

Day	n	GMT	CI 95%	SD	Min, max
N28	14	3,850	3,287-4,413	0,975	2,505;5,865
N180	14	2,198	1,604-2,792	1,028	0,425;4,666

Table 8: Comparison of solicited AEs between Viet Nam and Philippin's study

Solicited AEs		After N0 (n,%)	After N3 (n,%)	After N7 (n,%)	After N28 (n,%)
Redness	Viet Nam	10%	11,5%	11,5%	6,3%
	Magpantay et al.	38,4%	-	21,7%	3,9%
Pain	Viet Nam	6%	7,3%	6,3%	3,1%
	Magpantay et al.	1%	-	1,5%	0
Itching	Viet Nam	17%	17,7%	10,5%	4,2%
	Magpantay et al.	20,5%	23,9%	5%	10,5%

Supplementary

Table 1: Intensity of solicited local AEs after each injection

Intensity	After N0 (n,%)	After N3 (n,%)	After N7 (n,%)	After N28 (n,%)
1	35 (35%)	36 (37,5%)	27 (28%)	13 (13,5%)
2	1 (1%)	0	0	3 (3%)
3	0	0	0	0

Table 2: Intensity of solicited systemic AEs after each injection

Intensity	After N0 (n,%)	After N3 (n,%)	After N7 (n,%)	After N28 (n,%)
1	43 (43%)	25 (26%)	13 (13,5%)	12 (12,55%)
2	8 (8%)	3 (3%)	0	0
3	3 (3%)	0	0	0

Table 3: AEs intensity category

Intensity				
0	1	2	3	4
None	Not affected to daily activities	Affected to daily activity	Cannot perform daily activities (drug administration)	Visit medical facility or hospitalization