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RESEARCH PAPER

Immunogenicity and safety of inactivated chromatographically purified Vero cell-derived Japanese encephalitis vaccine in Thai children

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ABSTRACT

Summary: Inactivated mouse-brain-derived Japanese encephalitis vaccine has a worrisome safety profile and the live attenuated vaccine is unsuitable in immunodeficiency. This study aimed to evaluate the immunogenicity and safety of an inactivated chromatographically purified Vero-cell-derived JE vaccine (CVI-JE, Beijing P-3 strain) in children.

152 healthy Thai children, with an average (SD) age of 14.4 (3.8) months, received 3 doses of CVI-JE on days 0, 7–28, and one year. Homologous JE neutralizing antibody titers (NT) were measured. All subjects had seroprotection [geometric mean titer (GMT) 150] 28 days' post 2nd vaccination. The seroprotection rates at 1 year after primary series and and 1 month after the booster were 89.3% (GMT 49) and 100% (GMT 621), respectively. Local and systemic reactions–fever (17.6%), vomiting (8%), and poor appetite (5.3%)–were noted within 28 days' post-vaccination. All these symptoms were self-limited.

Conclusions: CVI-JE is safe, immunogenic, and provided high NT.

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Introduction

Japanese encephalitis (JE) virus is the most common cause of vaccine-preventable encephalitis in Asia. JE is endemic throughout most of Asia and parts of the western Pacific. The estimated annual number of clinical cases is 35,000–50,000, with mortality estimates ranging from 10,000 to 15,000 deaths (a case fatality rate of 20–30%); 30–50% of survivors suffer from neurologic and psychiatric sequelae.[1] Underreporting is common and some estimates predict up to 67,900 cases of JE per year.[2] Infections are transmitted through mosquitoes that acquire the virus from viremic animals, usually domestic pigs or water birds. Only about 1 in 250–500 infected individuals manifest clinical disease which is most prevalent among children aged <10 years.[3] Travel-associated JE, although rare, can occur among travelers to endemic areas.[4-5] There is no specific antiviral treatment for JE and vaccination is the single most important control measure. Currently, four major types of JE vaccine are available; (i) inactivated mouse-brain-derived vaccine based on either the Nakayama or Beijing strain, (ii) inactivated Vero-cell vaccine, based on SA 14-14-2 and Beijing strain (iii) live attenuated vaccine based on the SA 14-14-2 strain, and (iv) chimeric live attenuated vaccine, based on SA 14-14-2 strain.[6]

Live attenuated JE vaccines have limitations on their use in immunodeficient individuals or pregnant women, while the drawbacks of the inactivated mouse-brain-derived vaccine are its adverse events. Local and systemic adverse reactions to the inactivated mouse-brain-derived vaccine were reported in about 20% and 10% of vaccinees, respectively.[1] The serious systemic effects related to a severe allergic reaction such as urticaria, angioedema and respiratory distress were reported in 1–17 per 10,000 vaccinees.[7-8] and anaphylaxis was reported in a few cases.[9] Neuro-complications, which may be fatal, occurred in 1: 50,000–<1 million doses.[8-10] These neurological adverse events may be associated with the remnant of mouse brain tissue.

There are several inactivated Vero cell-derived JE vaccines. The vaccine containing the inactivated JE virus strain SA14-14-2 is IC51 or Ixiaro™ (Intercell Biomedical Livingston, UK). This vaccine has been registered in several regions such as Europe, North America, and Australia. In Japan, the inactivated JE virus strain Beijing-1 vaccines were produced by Biken (JEBIK V™) and the Chemo-Sero-Therapeutic Research Institute or Kaketsuken (ENCEVAC™) and have been used since 2009 and 2011, respectively.[11]

CVI-JE, the freeze-dried vaccine containing the inactivated JE virus strain Beijing P-3, produced by Liaoning Cheng Da Biotechnology Co., Ltd. (CVI-JE, JEVAC™) has been registered in China since 2008, and more than 7 million doses have been administered in China. The main pro-

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cesses of Vero cell viral culture, inactivation and purification of this vaccine are the same as the inactivated chromatographically purified Vero-cell rabies vaccine (Speeda™), which is produced by the same company. In brief, the method is a combination of micro-carrier bioreactor and perfusion culture. Perfusion culture is a dynamic culture system, which constantly feeds in fresh medium and pumps out waste medium without disturbing the cells in the bioreactor, thus providing a continuous nutritious environment during cell culture. The culture system also removes the toxic metabolites, reduces the accumulation of toxic metabolites from cell growth that may damage or affect virus antigen quality and helps to purify the product. The culture environment maintains a stable condition with low inhibition activity; therefore, it not only increases cell density, but also improves the refinement procedure. Column chromatography is an additional purification process.[12]

A study conducted in China in 375 children aged 8 months to 10 years given 2 doses on Day 0 and Day 7 proved that CVI-JE vaccine was safe and immunogenic. The seroconversion rate for the liquid formulation was 93.3% and adverse reactions were low-grade fever (0.8%), pain at the injection site (1.6%) and itching at injection site (1.1%). The seroconversion rate was 90.4% for freeze-dried formulation and the adverse reactions were low-grade fever (0.5%) and pain at the injection site (1.6%). These adverse reactions were mild and disappeared 72 hours' post-vaccination.[13]

The objective of our study is to evaluate the immunogenicity and safety of inactivated chromatographically purified Vero-cell-derived JE vaccine (CVI-JE, Beijing P-3 strain) among healthy children.

Results

One hundred and fifty-two eligible subjects were enrolled into the study from 3rd May 2010 to 10th August 2010. The male/female ratio was 1.08 (79/73). The average (SD) age was 14.4 (3.8) months (Table 1). All of the subjects received 1st CVI-JE vaccination according to the protocol without any immediate reactions. One subject withdrew from the study after the 1st dose of vaccine without any vaccine-related adverse reaction. One hundred and fifty-one subjects received the 2nd CVI-JE vaccination and had blood drawn. Blood collection for one subject was delayed (Day 80 after the 2nd vaccination) due to travel outside the study area. At 1 year, 148 subjects came for the follow-up visit. However, 3 of them were excluded due to receiving JE vaccine outside the study. The 3rd CVI-JE vaccination was given to 145 subjects. At 1 month after the 3rd vaccination, 145 subjects were revisited; however, blood was only taken from 144 subjects due to difficulties encountered drawing blood from one subject (Fig. 1).

Table 1. Demographic data of subjects who received CVI-JE.

Profile	Value
Number of subjects	152
Male: Female	79: 73
Age in months	
Minimum – Maximum	13 – 32
Average (SD)	14.4 (3.8)

Antibody responses

Five of 152 subjects had detectable neutralizing antibody (NT) against Japanese encephalitis virus on baseline blood (before vaccination on D0). By 28 days after the 2nd vaccination, all subjects had NT values higher than the protective level (100%). 130/145 (89.7%) subjects had existing NT values higher than the protective level at 1 year. When seropositive subjects on baseline blood were excluded, 125/140 (89.3%) subjects had NT values higher than the protective level at 1 year. All subjects had antibody titers above the protective level[14] at 28 days after the booster vaccination (Table 2).

The geometric mean titer (95% confidential interval) [GMT (95% CI)] after primary vaccination was 169.8 (138–209), which was much higher than the protective level (Fig. 2). The GMT (95% CI) at 1 year (before the booster vaccination) and 28 days after booster vaccination were 54.4 (42–70) and 648.9 (531–793), respectively. When those 5 seropositive subjects at baseline were excluded, the GMT (95% CI) after primary vaccination at 1 year and 28 days after booster vaccination were 150.0 (126–176), 49.3 (39–62), 621.7 (510–757), respectively (Fig. 2).

Among 5 subjects seropositive on Day 0, 4 had secondary immune responses, while 1 had primary immune response at 28 days after primary vaccination (Table 3).

Adverse events (AEs) after vaccinations

Regarding local AEs, 2 cases (0.5% of vaccination) had mild tenderness for 1 day, 2 cases (0.5% of vaccination) had redness (1 mild for 1 day after the 1st vaccination, 1 severe for 2 days after the 2nd vaccination), and 1 case (0.2% of vaccination) had mild ecchymosis for 8 days. All of the AEs appeared 1 day post-vaccination, except 1 case of tenderness appeared 2 days post-vaccination. No treatment was required for these local AEs (Table 4).

The most common solicited systemic AE was fever (axillary temperature $\geq$ 37.5°C) at 20.4%, 17.9%, and 14.5%, after each vaccination, respectively. The second and third most common AEs were vomiting and poor appetite. Four subjects each developed one episodes of urticarial following various doses of vaccine. All of these solicited AE were self-limited (Table 5). The most common unsolicited AE was upper respiratory tract infection (URI) (6.1%), followed by dermatitis (2.9%) (Table 6).

Twenty one serious adverse events (SAEs) were reported (12.7 episodes /100 person-year). All events were classified as SAEs due to hospitalization being required. The 3 major diagnoses were pneumonia, febrile convulsion, and acute gastroenteritis. Most SAEs occurred >1 month post-vaccination. Only 3/21 SAEs occurred within 1 month of vaccination, including 1 episode of asthma and 2 episodes of pneumonia (Table 7).

Discussion

The immunogenicity of CVI-JE evaluated by seroconversion rates (SCRs) and GMTs at 4 weeks after the 2nd and 3rd (booster) vaccination was excellent. The SCRs were 100% at 4 weeks after the 2nd and 3rd vaccination. The GMTs at 28 days after the 2nd and 3rd vaccination were 150 and 621.7,

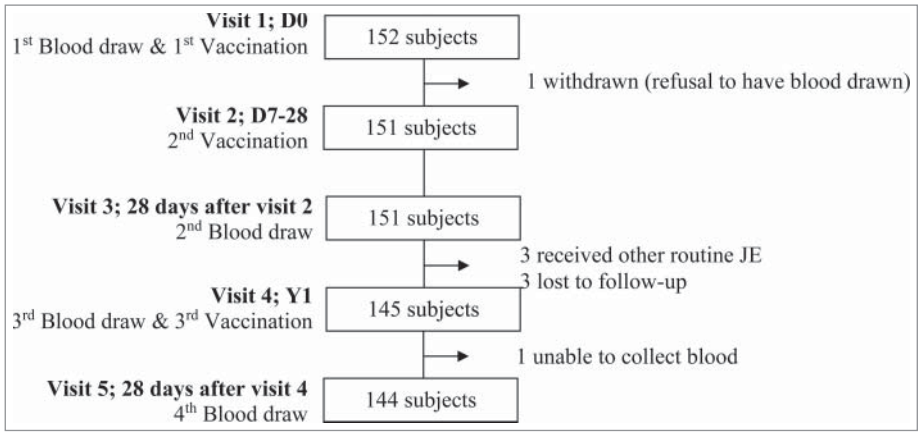

Figure 1. Disposition of subjects in the study.

respectively. The results are comparable with the study done in Chinese children by Libao Z, et al., which showed that the seroconversion rate was 90.4%.[13]

Two previous studies compared Vero-cell-derived inactivated JE vaccine (JE-VC) and JE-MB in children. A study in Indian children comparing IC51 (Ixiaro™) 2-dose schedule (3 and 6 μg on Days 0 and 28) and JE-MB 3-dose schedule of a JE-MB (on Days 0, 7, and 28) showed the SCRs on Day 56 after the last doses were 95.7, 95.2, and 90.9%, respectively, and the GMTs were 201, 218, and 230 respectively. No difference was seen in the safety profile between the vaccines.[15] The immunogenicity of a 3-dose vaccination [on Day 0, 14, and a booster dose (12 months after the 2nd dose)] of KD-287 (Encevac™) versus JE-MB (Nakayama strain) in Korean children was compared. The SCRs after the 2nd dose of KD-287 and JE-MB were 100% and 97.95% (GMTs 601, and 107) and after the 3rd dose were 100% and 98.9% (GMTs 13347, and 967) respectively. The two safety profiles were comparable but KD-287 had a higher incidence of fever after the 1st dose.[16]

The Vero-cell culture-derived inactivated JE vaccine (JE-VC) and JE-MB are thought to have similar properties, but the production process may be different. For instance, the inactivation and purification conditions for JE-VC may be better than those used for JE-MB. Electron microscopy showed that JE-MB contains virions with a somewhat smooth surface, whereas the surface of JE-VC virus particles is more similar to that of the native virus. Thus, the conformation of the E protein on the virus surface may be better conserved in JE-VC, which may be important for the superior immunogenicity of JE-VC.[11] Therefore, the immunogenicity of CVI-JE is comparable to other inactivated Vero-cell-derived JE vaccine, even though there is no direct comparison.

In this study, CVI-JE had no safety concern. Only a few solicited local adverse events were noted, together with a limited number of solicited systemic adverse events; this was comparable to the study conducted with Chinese children.[13] However, as there was no control group in this clinical study, whether these systemic adverse events were attributable to the study vaccine could not be determined. Most solicited and unsolicited adverse events monitored in this clinical study were not severe. Some adverse events might be due to common illnesses in this children's age group. 21 SAEs were reported, all of which were unlikely related to the study vaccine due to the time of occurrence and explainable cause. This may demonstrate a good safety profile for CVI-JE.

Vero-cell culture-derived inactivated JE vaccine is not only used in healthy individuals but WHO also recommends its use for special risk groups, such as immunocompromised persons, pregnant and lactating women.[17]

Although CVI-JE shows high GMTs after the 3rd dose, further study is necessary to determine long-term immunity and to confirm the efficacy and safety with large numbers of subjects for >1 year.

Three subjects received the booster JE vaccinations (inactivated mouse-brain-derived JE vaccine) outside the study. The NT raised was comparable with a secondary immune response after the booster vaccination.

This study's limitation is that it has no control, since JE-MB is used in the Thai Expanded Program in Immunization. Since this program uses a half dose of the vaccine in children aged 1–3 years, whereas CVI-JE use full dose in all age groups, it could not be compared for safety and immunogenicity when JE-MB was used as a control.

Conclusions

The chromatographically purified Vero-cell inactivated JE vaccine CVI-JE is safe and immunogenic. It resulted in 100% seroconversion and provided high GMTs after the primary doses and booster vaccination.

Table 2. Number (%) of subjects with NT >1/10 after primary (first 2 doses) and booster CVI-JE vaccinations.

	Subjects who had NT >1/10 (n/N, %)			
	Day 0	28 days after primary vac.	1 year	28 days after booster vac.
All subjects	5/152 (3.3)	151/151 (100)	130/145 (89.7)	144/144 (100)
Seronegative subjects on Day 0	0/147	146 (100)	125/140 (89.3)	139/139 (100)

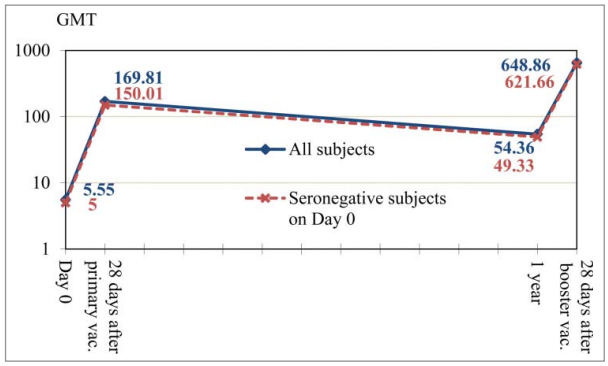

Figure 2. Geometric mean of NT (GMT) after CVI-JE.

Materials and methods

Study design: This was a clinical study with no control arm, performed in Bangkok, Thailand. The study was conducted in accordance with the protocol approved by the Ethical Review Committees for Research at the participating sites. This study is registered as a clinical trial with Clinical Trial registration number NCT01408537.

Subjects: The inclusion criteria for the study were (1) healthy Thai children aged 1–<3 years at the time of recruitment, (2) no previous history of JE vaccination, (3) available for all scheduled visits in the study period, and (4) written informed consent signed by a parent or guardian. The exclusion criteria were (1) known serious underlying disease, such as nervous system, heart, kidney, and liver disease, (2) known hypersensitivity to JE components, (3) previous history of JE infection, (4) received a blood component within the past 3 months, (5) known history of an immunocompromised condition, such as HIV/AIDs, malignancy, (6) under treatment with immunosuppressive drugs, such as systemic corticosteroid and/or anti-neoplastic drug, (7) febrile illness (temperature ≥ 37.5°C) or acute illness on the day of vaccination, (8) plan to leave the study area before the end of the study period, and (9) participation in another clinical trial. Written informed consent was obtained from each parent before study enrolment.

Vaccine: Freeze-dried CVI-JE (batch number 201001B01-4) is produced by Liaoning Cheng Da Biotechnology Co., Ltd. The excipients are human serum albumin and Dextran 40, PBS: q.s. as stabilizers. Adjuvant, antibiotics and thimerosal are not used. The

Table 3. Neutralizing antibody titers of 5 subjects seropositive at baseline.

	Neutralizing Antibody Titer				
	Day 0	28 days after primary vac.	1 year	28 days after booster vac.	Type of immune response
Subject 1	11	127	39	158	Primary
Subject 2	11	2,101	160	1,208	Secondary
Subject 3	861	63,760	15,383	24,100	Secondary
Subject 4	206	52,778	19,85	5,545	Secondary
Subject 5	1,168	30,431	2,034	1,732	Secondary

Table 4. Local adverse events within 7 days of each CVI-JE vaccination.

	Number (%)			
	1st dose (152 doses)	2nd dose (151 doses)	3rd dose (145 doses)	Total (448 doses)
Tenderness	Mild 1 (0.7)	Moderate 1 (0.7)	0	2 (0.5)
Redness	Mild 1 (0.7)	Mild 1 (0.7)	0	2 (0.5)
Ecchymosis	Mild 1 (0.7)	0	0	1 (0.2)

Tenderness:
mild: minor reaction when touched,
moderate: cries and protests when touched,
severe: cries when injected limb is moved or movement of injected limb is reduced.
Redness, Ecchymosis:
mild: diameter <2.5 cm,
moderate: diameter ≥2.5-<5 cm,
severe: diameter ≥ 5 cm

Table 5. Solicited systemic adverse events within 14 days of each CVI-JE vaccination.

	Number (%)			
	1st dose (152 doses)	2nd dose (151 doses)	3rd dose (145 doses)	Total (448 doses)
Fever				
Mild (axillary temp. 37.5–38.0°C)	18 (11.8)	9 (6.0)	8 (5.5)	35 (7.8)
Moderate (axillary temp.>38.0–39.0°C)	12 (7.9)	13 (8.6)	10 (6.9)	35 (7.8)
Severe (axillary temp. >39.0°C)	1 (0.7)	5 (3.3)	3 (2.1)	9 (2.0)
Chills				
Mild (1–2 episodes in a day)	1 (0.7)	0	1 (0.7)	2 (0.4)
Moderate (3–5 episodes in a day)	1 (0.7)	0	0	1 (0.2)
Poor appetite				
Mild (eating less than normal)	8 (5.3)	6 (4.0)	3 (2.1)	17 (3.8)
Moderate (refused 1–2 meals completely)	1 (0.7)	4 (2.6)	1 (0.7)	6 (1.3)
Severe (refused >2 meals completely)	0	1 (0.7)	0	1 (0.2)
Vomiting				
Mild (1 episode in a day)	15 (9.9)	10 (6.6)	6 (4.1)	31 (6.9)
Moderate (2–5 episodes in a day)	0	2 (1.3)	0	2 (0.4)
Severe (>5 episodes in a day)	1 (0.7)	1 (0.7)	1 (0.7)	3 (0.7)
Urticaria				
Present	1 (0.7)	2 (1.3)	1 (0.7)	4 (0.9)

Table 6. Unsolicited adverse events within 14 days of each CVI-JE vaccination.

		Number (%)			
Unsolicited AEs after	each vaccination	1st dose	2nd dose	3rd dose	Total
Respiratory tract	URI	9 (5.9)	12 (7.9)	6 (4.1)	27 (6.1)
	Bronchitis	0	2 (1.3)	1 (0.7)	3 (0.7)
	Pneumonia	0	1 (0.7)	0	1 (0.2)
Gastrointestinal tract	AGE	4 (2.6)	3 (2.0)	1 (0.7)	8 (1.6)
	Vomiting	0	0	1 (0.7)	1 (0.2)
Skin	Skin infection	0	0	1 (0.7)	1 (0.2)
	Dermatitis	12 (7.9)	1 (0.7)	0	13 (2.9)
	Itching	0	0	1 (0.7)	1 (0.2)
General	AFI	1 (0.7)	0	0	1 (0.2)
	Viral infection	0	0	1 (0.7)	1 (0.2)
	Chicken pox	0	2 (1.3)	0	2 (0.4)
	Viral exanthem	1 (0.7)	0	0	1 (0.2)
	HFM	1 (0.7)	0	1 (0.7)	2 (0.4)
Total		28 (18.4)	21(13.9)	13 (9.0)	62(13.8)

AFI = Acute febrile illness, AGE = Acute gastroenteritis,
HFM = Hand, foot and mouth disease, URI = Upper respiratory tract infection

Table 7. SAE throughout the study period.

Period since last vaccination (days)	Onset of SAE	Date of last vaccination before SAE (Dose)	Diagnosis
10	06 Jun 10	27 May10 (Vac2)	Asthma
12	16 Aug 10	04 Aug 10 (Vac2)	Pneumonia
15	28 Jun 11	13 Jun 11 (Vac3)	Pneumonia
33	17 Jul 10	14 Jun 10 (Vac2)	Pneumonia
58	07 Aug 10	10 Jun 10 (Vac2)	AGE
69	30 Aug 10	22 Jun 10 (Vac2)	AGE
74	27 Aug 10	14 Jun 10 (Vac2)	AGE
80	04 Sep 10	16 Jun 10 (Vac2)	Febrile convulsion
108	27 Aug 10	11 May 10 (Vac2)	Pneumonia
107	19 Nov 10	04 Aug 10 (Vac2)	HFM with febrile convulsion
140	18 Nov 10	01 Jul 10 (Vac2)	Suspected seizure*
160	25 Oct 10	18 May 10 (Vac2)	AGE
179	13 Nov 10	18 May 10 (Vac2)	Febrile convulsion
259	20 Apr 11	04 Aug 10 (Vac2)	Pneumonia
281	22 Mar 11	14 Jun 10 (Vac2)	Asthma
313	06 Jun 11	28 Jul 10 (Vac2)	Febrile convulsion
336	01 Jun 11	30 Jun 10 (Vac2)	Skin infection
345	02 Jun 11	22 Jun 10 (Vac2)	Dengue fever
349	23 May 11	08 Jun 10 (Vac2)	AFI
352	09 Jun 11	22 Jun 10 (Vac2)	URI
359	09 Jun 11	15 Jun 10 (Vac2)	Burn and AGE

AFI = Acute febrile illness, AGE = Acute gastroenteritis,
HFM = Hand, foot and mouth disease, URI = Upper respiratory tract infection
*Single episode without medical personnel confirmation (report by a parent)

corresponding potency is no less than that of the China's National Institute for the Control of Pharmaceutical and Biological Products (NICPBP) reference vaccine for a dose of 0.5 mL.

Procedures: Subjects were recruited at the Well Baby Clinic, Outpatient Department, Nopparat Rajathanee Hospital and the Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University. The subjects received CVI-JE subcutaneously. Two doses (0.5 mL/dose) for primary vaccination were given on Day 0 and 7–28 days later, and a booster vaccination was given at one year. Serological blood was drawn before the 1st vaccination dosage, 28 days after the second dose, at one year, but before booster vaccination and 28 days after the booster. Solicited and unsolicited adverse events were recorded within 14 and 28 days after each vaccination, respectively. Serious adverse events were recorded throughout the study period. There was no contraindication for other vaccines administration during the study period. However, there was no subject received other vaccine during 1 month after each study vaccine.

Determination of antibody response: Neutralizing antibody (NT) against Beijing P-3 strain was assayed using 50% plaque reduction neutralization test ($PRNT_{50}$) performed at the Center for Vaccine Development, Mahidol University, Thailand. The assay was performed using the method described by Russell et al.[18] Plaque count was determined by LLC-MK_2 plaque assay single overlay technique. For this technique, serum was thawed and heat-inactivated by incubation at 56°C for 30 minutes. Serial dilutions of serum were made (1:10, 1:100 and 1:1000). An equal volume of each diluted JE virus (Beijing- P3 strain), which contains about 40–60 plaque-forming units/ 0.2 mL, was added to each serum dilution tube. Following incubation at 37°C for 60 minutes, 0.2 mL of the mixture was removed from each tube and inoculated onto duplicate 6-well plates of confluent LLC-MK_2 cells. Each plate was incubated at 37°C for 90 minutes and the monolayer was then overlaid with 4 mL of 3.0% carboxymethylcellulose. Plates were incubated for 7 days at 37°C with 5% CO_2 followed by plaque count. The $PRNT_{50}$ titer was determined using the SPSS computer program. The percent reduction in plaque at each dilution level was plotted to determine the 50% reduction titer.

Definition

Detectable neutralizing antibody: NT against Japanese encephalitis virus ≥1/10 dilution.

Protective level: NT against Japanese encephalitis virus detectable at ≥1/10 dilution.

Seronegative: subject who had NT against Japanese encephalitis virus detectable at < 1/10 dilution.

Seropositive: subject who had NT against Japanese encephalitis virus detectable at ≥1/10 dilution.

Seroconversion rate (SCR): % seropositive after vaccination of seronegative subjects on D0.

Safety

The adverse events (AEs) information was daily collected for 14 days after each vaccination; it included solicited local AEs (7 days after each vaccination), solicited systemic AEs (14 days after each vaccination) and unsolicited AEs (14 days after each vaccination). The prelist of solicited local and systemic AEs was tenderness, redness, ecchymosis, fever, chills, poor appetite, vomiting, and urticaria.

Serious adverse event (SAE) information was collected throughout the study period.

The grading of AEs was defined as the following:

Tenderness:
mild: minor reaction when touched
moderate: cries and protests when touched
severe: cries when injected limb is moved or movement of injected limb is reduced

Redness, Ecchymosis:
mild: diameter <2.5 cm
moderate: diameter ≥2.5-<5 cm
severe: diameter ≥ 5 cm

Fever:
mild: axillary temp. 37.5–38.0°C
moderate: axillary temp.>38.0–39.0°C
severe: axillary temp. >39.0°C

Chills
mild: 1–2 episodes in a day
moderate: 3–5 episodes in a day
severe: >5 episodes or almost of the day

Poor appetite
mild: eating less than normal
moderate: refused 1–2 meals completely
severe: refused >2 meals completely

Vomiting
mild: 1 episode in a day)
moderate: 2–5 episodes in a day
severe: >5 episodes in a day

Urticaria
no grading

Statistical analysis

Descriptive analysis was used to describe the proportion of subjects who had seroconversion [NT > 1/10 in subjects who were seronegative (NT <1/10) at baseline] and the geometric mean titers (GMT) at 28 days after primary and booster vaccination and at 1 year after primary vaccination. The percentages of solicited and unsolicited, local and systemic adverse events and serious adverse events were calculated. The sample size for the study was calculated based on the immunogenicity data from a previous study. It was hypothesized that the antibody response among Thai children would be the same as the previous study among Chinese children.13 (seroconversion rate of 90%). With 95% confidence level, 80% power, the worst seroconversion rate that the difference could be detected was 85%; sample size was 138. With compensation for possible 10% loss to follow-up, and 1% seropositivity at baseline; therefore, the actual sample size was 152.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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