

Immunogenicity of Low Dose Japanese Encephalitis Vaccine (BIKEN) Administered by the Intradermal Route : Preliminary Data

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Japanese encephalitis (JE) is a widespread mosquito-borne flavivirus infection that occurs in annual epidemics of potentially lethal encephalitis in Thailand.¹ In northern Thailand, more than 90% of children have neutralization antibody by age 14; 50% seroprevalence is found to be age-related.⁵ In an ongoing, placebo-controlled study of the efficacy of an available inactivated Japanese encephalitis vaccine (JEVAC;BIKEN), greater than 90% protection was afforded to volunteers, aged 1-4 years, who received two 0.5 ml (age 3) or two 1 ml (other ages) doses subcutaneously (SC). Partly on the basis of this work, the Ministry of Public Health has now approved the sale and use of this product in Thailand (Tuchinda P personal communication).

This effective and safe vaccine, prepared from formalin inactivated Japanese encephalitis virus (JE) grown in suckling mouse brains and purified by precipitation and gradient centrifugation, is not yet widely available in Thailand. Its use is expected to be limited due to its high cost for a two dose primary immunization.

Two other recently developed

SUMMARY Two hundred twenty-four immune and non-immune adults were systematically assigned to receive a single dose of Nakayama strain JEVAC in one of four study "arms" : 0.1 ml ID, 0.2 ml ID (injection of 0.1 ml at two sites), 0.3 ml ID (injection of 0.1 ml at three sites), or 1.0 ml SC. Immune responses after this single dose (in many cases "booster") was assumed to reflect immune responses of a primary series and was assessed qualitatively (percent seroconversion) and quantitatively (geometric mean titer) a 30 and 90 days post immunization. The results showed that JEVAC given 0.1 ml. ID at two sites is likely to be as immunogenic as 1.0 ml. given SC.

viral vaccines, hepatitis B vaccine and human diploid rabies vaccine, similarly are too expensive for effective wide scale utilization but controlled studies have shown these vaccines are equally immunogenic when administered at one-tenth dose intradermally (ID).²⁻⁸

The objective of this protocol was to compare the immunogenicity of JEVAC (BIKEN) given at a reduced dose ID to that of the same vaccine given as two standard doses SC. Formally stated, the two hypotheses being proposed for testing are:

1. $GMT_{ID} = GMT_{SC}$
2. $\% \text{ seroconversion}_{ID} = \text{seroconversion}_{SC}$

MATERIALS AND METHODS

Volunteers

Healthy 224 adult of both sexes, who lived in Chiang Rai province were divided randomly into 4 groups without statistical differences in age and sex (Table 1):

group 1. 54 volunteers received 0.1 ml ID

group 2. 58 volunteers received 0.2 ml ID (0.1 ml at two different sites)

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Table 1 Age and sex ratio of the four dose groups, intradermal JEV vaccine pilot study.

Dose	No.	Mean age (½ SD)	M:F Ratio
0.1 ml ID	53	32.9 ½ 8.0	1:4.4
0.2 ml ID	58	31.3 ½ 7.6	1:2.6
0.3 ml ID	56	33.1 ½ 8.1	1:2.1
1.0 ml SC	56	32.5 ½ 8.5	1:3.7

Table 2. Immune status of the four dose groups, intradermal JEV vaccine pilot study at day 0, day 30 and day 90.

Pre-bleed PRNT ₅₀ status	Dose	No. evaluated	Log JEV PRNT ₅₀		
			day 0	day 30	day 90
Negative	0.1 ml ID	27	0	1.24 ± 1.59	0.73 ± 1.38
	0.2 ml ID	40	0	1.24 ± 1.48	1.24 ± 1.24
	0.3 ml ID	29	0	1.40 ± 1.71	1.11 ± 1.26
	1.0 ml SC	33	0	1.40 ± 1.71	1.59 ± 1.48
Positive	0.1 ml ID	27	2.26 ± 0.69	3.26 ± 0.68	2.65 ± 0.59
	0.2 ml ID	18	2.06 ± 0.41	3.36 ± 0.57	2.76 ± 0.28
	0.3 ml ID	24	2.15 ± 0.38	3.66 ± 0.93	2.97 ± 0.78
	1.0 ml SC	23	2.04 ± 0.43	3.57 ± 0.48	2.97 ± 0.28

group 3. 56 volunteers received 0.3 ml ID (0.1 ml at three different sites)

group 4. 56 volunteers received 1 ml SC.

Analysis of variance of mean age by groups, stratified by PRNT₅₀ or not, found no significant difference ($p > 0.05$).

Vaccine

Lyophilized, inactivated, monovalent JEVAC (BIKEN) reconstituted with sterile water, was administered, by a nurse trained to give ID injections, using a 1 ml tuberculin syringe and a 26 gauge needle into the deltoid (SC) or volar forearm (ID). Satisfactory ID technique was determined by an investigator who recorded the presence or absence of a wheal.

Specimens

Whole blood, collected in one 5 ml tube was centrifuged and the serum collected and stored in a pre-labeled tube at -20°C until the time of assay. Sera for serology were collected at days 0, 30 and 90 and tested for JEV neutralizing antibody.

Serology

Neutralizing antibody was measured by a standard 50% plaque reduction neutralization assay (PRNT)¹⁵ adapted for LLC-MK₂ monolayers grown in 24-well plates incubated in 5% CO₂. PRNT titers less than 1:10 were considered non-immune. Seroconversion was defined as a 4-fold titer increase or a change from a titer of < 1:10 to ≥ 1:10

Qualitative measure

Response defined as a NT rise ≥ 4-fold, ie from < 1:10 to ≥ 1:20 or ≥ 4 x the titer on day 0, at either day 30 or day 90.

Quantitative measure

Geometric mean titer of responders, at day 30, or GMT of cumulative responders (response at either day 30 or day 90).

RESULTS

221 serum samples collected on day 0 (pre-bleed) were divided into two groups:

1) 78 seroconversion after vaccination out of 92 immune persons (Table 2).

Table 3 95% confidence limits for response measured at 90 days.

Pre-bleed PRNT ₅₀ status	Dose	No. responses/No. evaluated	95% CI
Negative	0.1 ml ID	11/27 (41%)	22%-61%
	0.2 ml ID	23/40 (58%)	41%-73%
	0.3 ml ID	15/29 (52%)	33%-71%
	1.0 ml SC	18/33 (55%)	36%-72%
Positive	0.1 ml ID	18/27 (67%)	46%-83%
	0.2 ml ID	15/18 (83%)	50%-96%
	0.3 ml ID	24/24 (100%)	100%
	1.0 ml SC	21/23 (91%)	72%-99%

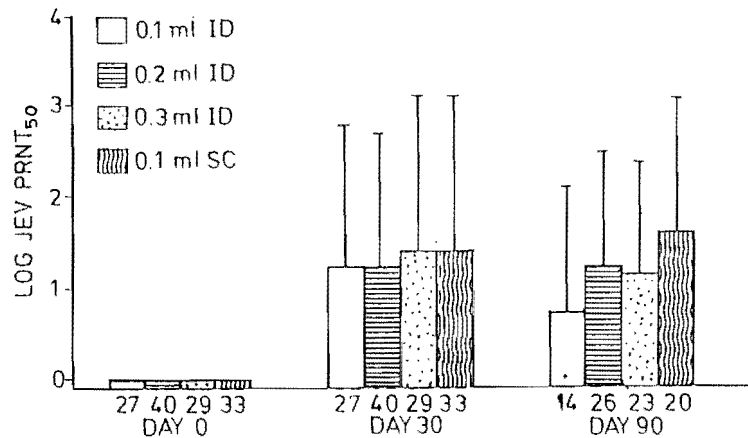


Fig. 1. Geometric mean titer JEV PRNT₅₀ of 4 different doses, pre-bleed PRNT₅₀ status negative on day 0.

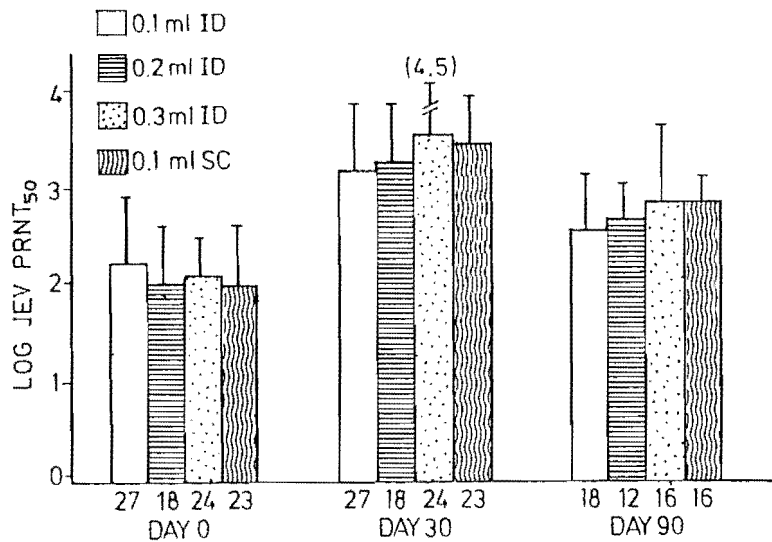


Fig. 2 Geometric mean titer JEV PRNT₅₀ of 4 different doses, pre-bleed PRNT₅₀ status positive on day 0.

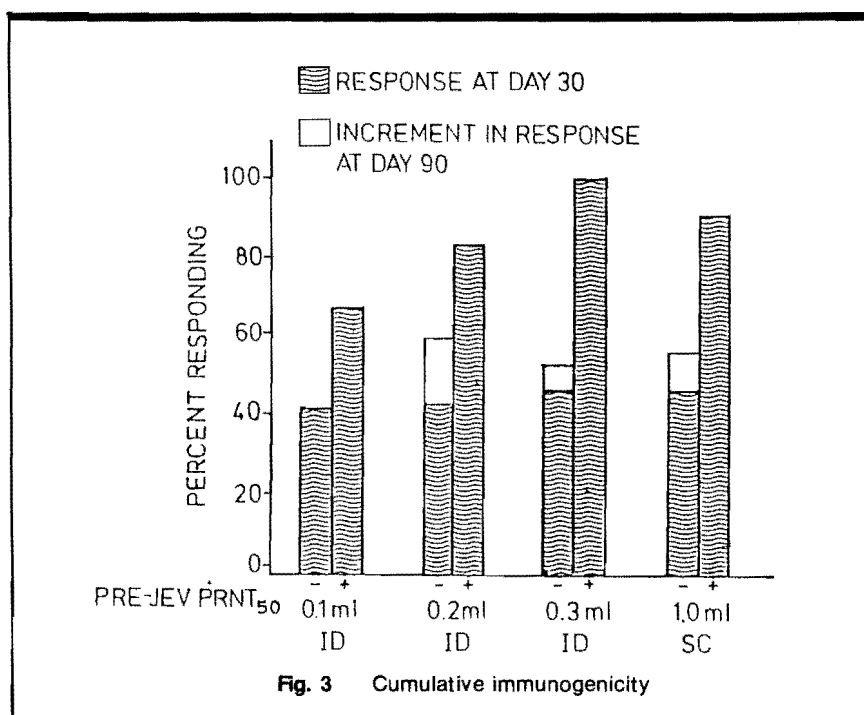


Fig. 3 Cumulative immunogenicity

2) 67 seroconversion after vaccination out of 129 non-immune persons.

The results compare each group according to GMT in Table 2, Fig. 1 and Fig. 2. The analysis of seroconversion rate in 2 groups is shown in Table 3 and comparative analysis of cumulative immunogenicity in previously non-immune and immune vaccine on day 30 and day 90 is shown in Fig. 3.

DISCUSSION

Inactivated Japanese encephalitis vaccine (JEVAC) has been found to be highly effective in preventing encephalitis due to Japanese encephalitis virus in Thailand.^{9,11} Because of the widespread nature of the virus and mosquito vector, a national immunization campaign is being considered.^{10,12} The record of successful intradermal (ID) immunization with hepatitis B vaccine as a cost reduction strategy prompted us to assess the immunogenicity of JEVAC (ID) in volunteers. Two hundred twenty four adults were sys-

tematically assigned to receive a single dose of Nakayama strain JEVAC is one of four study "arms": 0.2 ml ID (injection of 0.1 ml at two sites), 0.3 ml ID (injection of 0.1 ml at three sites), or 1.0 ml SC. Immunogenicity after this single dose (in many cases "booster") was assumed to reflect immunogenicity of a primary series and assessed qualitatively (percent seroconverting) and quantitatively (geometric mean titer) at 30 and 90 days post immunization. Volunteers were stratified by prevaccine JE neutralization titer (NT) as non-immune (NT < 1:10) or immune (NT ≥ 1:10).

Geometric mean titers, compared between the SC group and each ID group examined by one-way analysis of variance according to drug regimen, were not different, though the sample size is not large enough to ascertain equivalence statistically. Given these preliminary data, we believe JEVAC given 0.1 ml ID at two sites is likely to be as immunogenic as 1.0 ml given SC. The jet injector using special heads

may be used safely for ID vaccination. The hypothesis of equivalence of immunogenicity for the ID and SC routes should be tested in young children. If sustained, an 80% savings in vaccine cost could be achieved.

Assumptions of the study

1. PRNT₅₀ procedure measures little cross-reactive dengue antibody.
2. Immunogenicity of a single dose of vaccine in presumably immune subjects is proportional to the 2 or 3 doses immunogenicity of the same vaccine in non-immune subject.
3. The experiment was conducted during the "JE season" (June-September 1987). Each group had an equal number of natural "boosters" from the bite of JEV-infected mosquitos.

Criticism of the study

The small sample size employed results in large 95% confidence limits for response rates. While all regimens were immunogenic, the actual equivalence of 0.1 ml ID and 1.0 ml SC cannot be confidently assumed.

Conclusions

1. "Biken" JEV vaccine is immunogenic when administered either ID or SC to adults previously infected with JEV.
2. Large differences between the mean qualitative or quantitative measurement of immunogenicity at 30 days post immunization were not found. At 90 days; in those that had negative JEV PRNT₅₀ assays at the outset, the GMT had begun to decline (the significance of this is unknown).
3. Given these limited data, we propose that 0.1 ml ID delivered at two sites may be equivalent to 1.0 ml SC.

Recommendation

A study of sufficient size to be able to detect a 10% difference in

immunogenicity should be carried out in young children (1-3 years old) using two doses of either 0.1 ml ID or 0.5 ml SC, at one month intervals. If a 65% response rate for both routes is assumed, this study would require 900 volunteers. A finding of equivalence would support a strategy to reduce the cost of immunization by 80% without seriously compromising efficiency of delivery.

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