

Short-Term Safety of Live Attenuated Japanese Encephalitis Vaccine (SA14-14-2): Results of a Randomized Trial with 26,239 Subjects

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The short-term safety of an effective and inexpensive new live attenuated Japanese encephalitis vaccine (SA14-14-2) was studied in a randomized trial, using block randomization. Of 26,239 children who were enrolled, half received the vaccine and half served as controls. Subjects were prospectively followed for 30 days for severe adverse events, such as encephalitis, meningitis, and "all-cause" hospitalization. No cases of encephalitis or meningitis occurred in either group. The upper 95% confidence limit for adverse events not occurring among subjects receiving their first dose was 4.1/10,000. Risk ratios and 95% confidence intervals for other adverse events were 0.70 (0.43–1.15) for all-cause hospitalization, 0.91 (0.37–2.22) for seizure, and 0.79 (0.56–1.11) for fever lasting ≥ 3 days. These data attest to the short-term safety of the SA14-14-2 virus strain and the hamster kidney cell substrate.

Japanese encephalitis (JE) is an important public health problem throughout a vast region of Asia. Conservative estimates place the annual incidence at $>35,000$ cases, mostly in children [1]. Among these, $>10,000$ die of JE, and an equal number develop permanent neurologic sequelae. JE is caused by a flavivirus that circulates in zoonotic cycles involving many vertebrate species and is transmitted to humans by the bite of several mosquito species. Because of its zoonotic cycle, prospects for eradicating JE from the environment are dim, and universal childhood vaccination is likely to remain essential for its control in the foreseeable future.

A killed mouse brain-derived JE vaccine with 91% efficacy (95% confidence interval [CI], 70%–97%) [2] that is manufactured by Biken (Osaka, Japan) is available internationally, although in insufficient quantities to meet the need worldwide. Similar mouse brain-derived vaccines are produced in limited quantities by manufacturers in other countries. The price of Japanese-produced JE vaccine in Asia is about US \$5/dose,

with a three-dose primary vaccination series recommended and yearly boosters administered in some countries. Thus, the expense and inconvenience of mouse brain-derived JE vaccine hinder immunization efforts. Adding to this difficulty has been the occurrence of rare hypersensitivity and neurologic reactions, including encephalitis and encephalopathy, that have been associated temporally (although not necessarily causally) with the existing vaccine [3].

In 1988, an inexpensive (US \$.75/dose) live attenuated primary hamster kidney-derived JE vaccine (SA14-14-2) was licensed in China [4]. Prior to licensure, trials conducted in highly endemic areas indicated 95% efficacy after a single dose [1, 5]. A recent case-control study conducted in an area less endemic for JE showed 80% effectiveness (95% CI, 44%–93%) after one dose and 97.5% effectiveness (95% CI, 86%–99.6%) after two doses administered 1 year apart [6].

Some information concerning the safety of this vaccine is known. Results of a nonexperimental cohort study of the candidate vaccine were published in the Chinese literature [5]. Although the study lacked methodologic detail, it showed that no serious events were detected among 588,512 vaccinees. The vaccine's safety was also studied among 1026 children (5–12 years old) who were followed for 14 days after receiving their first dose of this vaccine. No cases of encephalitis or other serious adverse events were observed [4]. The present trial was done to complement earlier data with a more formal study of the 30-day safety of the SA14-14-2 virus strain and of hamster kidney cells as a substrate for the production of live attenuated vaccines.

The goals of the current study were to identify and measure the incidence of severe adverse events occurring up to 30 days after immunization and to identify and measure the incidence

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of common mild events occurring within 7 days of inoculation with this vaccine.

Methods

Study design. The randomized trial used health centers as the unit of randomization. Subjects were assigned to receive or not receive vaccine and were prospectively followed for 30 days for serious adverse events, such as encephalitis, meningitis, and "all-cause" hospitalization. In addition, a convenience subsample of vaccinated subjects was followed more closely for 7 days in order to identify mild adverse events.

Setting. The study was done in Chengdu, the capital of Sichuan Province, People's Republic of China. Children in Chengdu are vaccinated with JE vaccine at approximately ages 1, 2, and 6 years during campaigns occurring each spring. In response to announcements regarding vaccination campaigns for a particular year, children are brought to neighborhood health centers, which provide primary health care, including vaccinations. No other vaccine is administered concurrently.

Study population. Study subjects included all children who presented for JE immunization to one of the participating health centers during the 1995 vaccination campaign and who possessed a vaccination card indicating no or one previous dose of JE vaccine. Specific health centers were selected for enrollment on the basis of the number of children served and the willingness of center staff to participate. Health center workers were trained and given written instructions on study procedures. A sufficient number of centers was recruited to enroll at least 10,000 exposed and 10,000 unexposed children so that adverse events as rare as 3/10,000 could be detected [7].

Randomization. Participating health centers were randomized to either treatment or control status in the following manner. First, centers were pair-matched within each of the five urban districts (based on the neighborhood and the number of children served by each center). Smaller centers were combined with two or three others for purposes of randomization. Then, for each pair, a drawing was done to randomly assign one center to treatment status and the other to control status.

Interventions. Children presenting to either type of health center underwent an initial evaluation that consisted of an examination of their vaccination card and a structured interview with their parent or caretaker. Children presenting to treatment centers were then vaccinated at this initial visit, while children presenting to control centers had their vaccination delayed until after the observation period. The vaccine was the same one that is routinely used in those health centers, and it was manufactured, as described elsewhere [1], by the Chengdu Biological Products Institute (Chengdu).

Outcome measures. All illnesses prompting a health center visit during the study period were recorded, including the pediatrician's diagnosis. In addition, subjects and parents from both groups were asked to return to the center 30 days after the enrollment visit. Parents then underwent a structured interview regarding hospitalizations and illnesses that occurred since the initial visit. Children in the control group were then vaccinated. In an intensive effort to attain complete follow-up, study personnel made home visits to subjects who did not return after 30 days.

To evaluate the parents' recall of hospitalizations, we implemented a surveillance system in the five to six largest hospitals of each of the five study districts. Investigators visited each hospital twice per week and recorded the name and address of all children who were admitted and who were 1–3 years old. They compared this list with the roster of study subjects. Ninety-eight percent of all admissions ascertained through the surveillance system were also reported by the parent, and all hospitalizations discovered by either method are reported here. Because of the large number of small hospitals in Chengdu, admissions to surveillance hospitals accounted for only 48% of all study admissions.

Medical records for all hospitalized subjects were examined by abstractors who were blinded to the study group. Because the primary outcomes of interest (encephalitis and meningitis) are severe events that should reliably result in hospitalization, these outcomes were defined on the basis of a physician diagnosis. Bronchitis was defined on the basis of a physician diagnosis from a hospital admission or health center visit. Other outcomes (severe reactions consistent with anaphylaxis, seizure, fever lasting ≥ 3 days, diarrhea, and upper respiratory infection) were diagnosed on the basis of a parent report or a physician diagnosis.

A convenience sample of 266 vaccinated subjects was enrolled for a more intensive evaluation consisting of a brief physical examination performed by a study pediatrician at days 1, 2, 3, and 7 after vaccination.

Statistical analysis. Risk ratios and 95% CIs that accounted for clustering by health center were calculated by use of the CSAMPLE component of Epi Info (version 6.04; Centers for Disease Control and Prevention, Atlanta) [8]. Epi Info was also used to calculate exact mid-P 95% CIs [9] for the incidence of adverse events among vaccinees.

Results

Of the 180 participating health centers, 104 were assigned treatment status and 76 were assigned control status. There were 26,239 eligible subjects, with 13,275 of these presenting to treatment centers and 12,964 presenting to control centers. The groups were well-balanced with respect to prevaccination factors: The mean (SD) age was 1.9 (0.6) years, 52% of subjects were boys, and 55% were due to receive their first JE vaccination.

The mean (SD) duration of follow-up in the vaccinated group was 30.1 (1.5) days, and in the unvaccinated group, it was 30.1 (1.2) days. Loss to follow-up occurred in only 9 exposed subjects and 12 unexposed subjects, for a total of 21 losses to follow-up (8/10,000). Thirteen of these subjects were known to have moved out of Chengdu and could not be located. There was one death, which was due to an automobile accident that occurred in an unvaccinated subject.

The frequency of adverse events observed within 30 days of vaccination is presented in table 1. None of the events of primary interest (encephalitis, meningitis, or all-cause hospital admission) occurred more frequently in the vaccinated group than in the unvaccinated group. These results did not differ when subgroups were analyzed on the basis of whether the

Table 1. Number (%) of subjects with complete follow-up who experienced adverse events in the 30 days following immunization with JE vaccine.

| Event | Vaccinated group (n = 13,266) | Unvaccinated group (n = 12,951) | Risk ratio (95% confidence interval)* |
|---|----------------------------------|------------------------------------|--|
| Encephalitis | 0 (0.0) | 0 (0.0) | Undefined |
| Meningitis | 0 (0.0) | 0 (0.0) | Undefined |
| Hospital admission | 82 (0.6) | 114 (0.9) | 0.70 (0.43–1.15) |
| Severe reaction consistent with anaphylaxis | 0 (0.0) | 0 (0.0) | Undefined |
| Seizure | 14 (0.1) | 15 (0.1) | 0.91 (0.37–2.22) |
| Fever lasting ≥ 3 days | 357 (2.7) | 442 (3.4) | 0.79 (0.56–1.11) |
| Diarrhea | 12 (0.1) | 11 (0.1) | 1.06 (0.46–2.49) |
| Upper respiratory infection | 292 (2.2) | 353 (2.7) | 0.81 (0.55–1.18) |
| Bronchitis | 38 (0.3) | 44 (0.3) | 0.84 (0.49–1.44) |

* Accounts for clustering by health center [8].

vaccine administered was the first or second dose (data not shown). No cases of encephalitis, meningitis, or severe systemic reaction consistent with anaphylaxis were observed in either group. Therefore, the point estimate for the incidence of each of these adverse events (and other event types that did not occur) among vaccinees was 0%. The upper 95% confidence limit (CL) for the incidence of these events in the vaccinated group overall (i.e., children receiving the first or second dose; $n = 13,266$) was 2.3/10,000. Among children receiving their first dose ($n = 7262$), the upper 95% CL was 4.1/10,000. Among children receiving their second dose ($n = 6,004$), the upper 95% CL was 5/10,000.

Evidence of adverse events was also sought by means of physical examination in 266 vaccinated subjects at days 1, 2, 3, and 7 after immunization. Similar to the case with the overall study population, the mean (SD) age in this subgroup was 1.9 (0.6) years, and 53% were boys. The adverse event data from this subgroup are presented in table 2. Fever was the most common adverse event, occurring in 13 subjects (4.9%), which was consistent with the incidence of prolonged fever in the overall vaccinated population (2.7%). Irritability was the second most common event, occurring in 10 subjects (3.7%). One subject was hospitalized with a diagnosis of pneumonia. No other serious events were observed in this group.

Discussion

This study provides convincing evidence of the 30-day safety of a live attenuated JE vaccine. In this large randomized trial, the incidence of adverse events in the exposed group was no higher than that of a concurrent control group. Further, because no cases of encephalitis, meningitis, or anaphylaxis occurred, the point estimate for the incidence of each of these events

was 0%, with an upper 95% CL for the pooled incidence among first- and second-dose recipients of 2.3/10,000. Because children receiving their second dose might be at a lower risk of vaccine-induced JE (because of immunity acquired from the first dose), the incidence of encephalitis among children receiving their first dose is a more conservative measure of the vaccine's safety. The point estimate of the incidence of encephalitis in this group is also 0%, with an upper 95% CL of 4.1/10,000.

These results need to be interpreted in light of concerns common to all epidemiologic studies. Selection bias and confounding have been largely avoided because of random assignment, albeit by block randomization. Because hospitalization is a highly memorable event for the parents of a young child, we believe that underascertainment of outcomes based on parents' failure of recall is unlikely. This belief is strengthened by the observation that 98% of all hospital admissions detected through the surveillance system were also reported by parents. Further, if underascertainment did play a role, we believe that in this unblinded study, parents would be more likely to remember adverse events that occurred in vaccinated subjects than those that occurred in unvaccinated subjects, which would bias the study results against the vaccine.

This candidate live attenuated JE vaccine is known to be effective [1, 6], and previous evidence attesting to its short-term safety [4, 5] has now been confirmed in this large randomized trial. Thus, the short-term safety of both the SA14-14-2 virus strain and of hamster kidney cells as a substrate for the manufacture of live attenuated vaccines has been well-documented.

In summary, we have conducted a randomized trial in 26,239 children to evaluate the short-term safety of a live attenuated JE vaccine. The results indicate that the vaccine is not associated with an increased incidence of encephalitis, meningitis,

Table 2. Adverse events observed in a convenience sample of 266 vaccinated subjects examined at days 1, 2, 3, and 7 after JE immunization.

| Event | No. of subjects experiencing event (n = 266) | % (95% confidence interval) |
|---------------------------------|--|--------------------------------|
| Fever $\geq 37.5^\circ\text{C}$ | 13 | 4.9 (2.7–8.2) |
| Hives | 1 | 0.4 (0.02–1.8) |
| Angioedema | 0 | 0 (0–1.1) |
| Joint swelling | 0 | 0 (0–1.1) |
| Rash | 6 | 2.2 (0.9–4.6) |
| Asthma | 0 | 0 (0–1.1) |
| Cough | 9 | 3.4 (1.6–6.1) |
| Injection-site tenderness | 1 | 0.4 (0.009–2.1) |
| Axillary adenopathy | 0 | 0 (0–1.1) |
| Irritability | 10 | 3.8 (1.9–6.6) |
| Vomiting | 3 | 1.1 (0.3–3.0) |
| Diarrhea | 2 | 0.8 (0.12–2.5) |
| Seizure | 0 | 0 (0–1.1) |

all-cause hospitalization, or other serious adverse events in the 30 days following vaccination. This provides reassuring evidence of the short-term safety of an affordable vaccine with known effectiveness.

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