The Immunogenicity and Safety of the Live-attenuated SA 14-14-2 Japanese Encephalitis Vaccine Given with a Two-dose Primary Schedule in Children

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INTRODUCTION

Japanese encephalitis (JE) virus is the leading cause of viral encephalitis in Asia. The recent emergence of JE virus in new territories that has been attributable to the climate change and international travel has raised the concern of global public health (1, 2). Because there are still limitations in vector control and specific antiviral treatment of JE, vaccination is the most effective strategy for prevention.

Although the inactivated Vero-cell culture JE vaccine has recently introduced, two JE vaccines have been widely used in Korea: the mouse brain-derived, killed-inactivated JE vaccine (MBDV) and the live attenuated SA 14-14-2 JE vaccine (LajeV). MBDV was first developed in Japan (BIKEN, distributed by Sanofi Pasteur as JE-VAX) and the only commercially used vaccine worldwide for past decades (3, 4). Local productions of similar vaccine have been used effectively to immunize travelers and residents in enzootic areas, and this has reduced JE incidence in some countries. However, the MBDV had the uncertainty as to the duration of protection and required multiple doses to maintain immunity (5, 6). In addition, an unacceptable occurrence of neurologic and hypersensitivity reactions has prompted a major manufacturer to cease production in Japan (6-8). In 1994, two cases of death temporally associated with MBDV led to a serious social issue, and to establish the Vaccine Injury Compensation Program in Korea. As such, there has been an apparent need for a vaccine with an enhanced safety profile and smaller dose schedule to protect against JE, which led to the introduction of LajeV.

Since its licensure in 1988, more than 300 million doses of LajeV have been administered in China where it has shown an excellent efficacy and safety profile (9, 10). This vaccine manufactured in China has been used in countries of western pacific region and Asia (Nepal, Sri Lanka, India, and Korea), and constituted more than 50% of the global production of all JE vaccines in 2005 (11). However, the lack of precedence for using a primary hamster kidney cell line as the substrate and the safety profile remain special issues of concern (12). Nevertheless, some physicians prefer to use the LajeV because of simple schedule compared with MBDV. The primary series in Korea is scheduled with two doses administered 12 months apart in infants aged 1-2 yr. This preferred interval was established based only...
on data from studies conducted in China (13), and no published data for the primary series using a 12-month interval exists for countries other than China. Therefore, this study was undertaken to assess the immunogenicity and safety of LAJEV according to the local schedule in Korea.

MATERIALS AND METHODS

Study design
The study was designed as an open-label, prospective cohort, multicenter trial in Korea. The study was performed at five centers between July 2, 2007 and October 26, 2009. At enrollment, eligible participants from 1 to 3 yr of age were given a brief health examination, blood samples were taken, and the first vaccine dose was administered. A follow-up visit occurred at 4-6 weeks after vaccination, at which point blood samples were collected from all children. One year after the first vaccination, a blood sample was collected and the second vaccine dose was administered. As with the first dose, a follow-up visit was done at 4-6 weeks after second vaccination and blood samples for immunogenicity analysis were collected.

Study subjects
Healthy children without significant medical history or clinically significant physical examination or laboratory findings at screening were eligible. Exclusion criteria included: congenital or acquired immunodeficiency or immunosuppressive conditions; hypersensitivity to any of the vaccine components; use of systemic corticosteroids, immunosuppressive medications, or biological agents in the previous 3 months; or anticipated administration of such medications during the course of the study; a known history of clinically, serologically or microbiologically confirmed flavivirus infection; or febrile or acute illness on the day of injection. The protocol allowed children to receive additional vaccines as recommended, including hepatitis A vaccine at 12 to 24 months of age, *Haemophilus influenzae* type b conjugate vaccine and pneumococcal conjugate vaccine at 12 to 15 months of age, and diphtheria, tetanus, acellular pertussis (DTaP) vaccine at 15 to 18 months of age, as long as doses were given at least 8 days after and at least 4 weeks before a dose of the study vaccine. Measles, mumps, rubella (MMR) and varicella vaccines were allowed if administered at least 4 weeks before or after administration of the study vaccine.

Study vaccine
LAJEV (CD-JEVAX™, Chengdu Institute of Biological Products, Chengdu, China) was used in this study. The main component of this vaccine is SA14-14-2 strain of JE virus. The others are lactose, sucrose, gelatin, human blood albumin and urea. Each subject was administered 0.5 mL of freshly reconstituted vaccine subcutaneously to the upper arm as instructed by the manufacturer.

Immunogenicity evaluation
Blood samples for immunogenicity assays were taken prior to and 4-6 weeks after each vaccination. The samples were kept frozen at -70°C and were sent to the central laboratory of the Korean National Institute of Health. Sera were tested for neutralizing antibodies using the plaque reduction neutralization test (PRNT) as followings. Test sera were heat-inactivated at 56°C for 30 min and diluted 1:5 and in serial two fold dilutions (up to 1:2,560). The Nakayama strain (heterologous SA-14-14 strain) was diluted in phosphate buffer saline containing 5% fetal calf serum and 5% guinea pig complement to provide 200 pfu/0.1 mL, and added in equal volume to each serum dilution. Serum-virus mixtures were incubated over-night at 48°C and added to drained baby hamster kidney 21 cell culture monolayers grown in six well plates. After adsorption for one hour at 37°C, the monolayers were overlaid with semisolid medium. Approximately 5 days later, when plaques could be seen microscopically, the medium was removed. The complete titration of each serum was carried out in a single test (14). The end point for neutralization was the highest dilution of serum reducing plaques by 50% of the JE challenge virus, compared with a negative serum control. JE neutralizing antibody titer ≥ 10 was considered seropositive and seroprotective (15).

Safety evaluation
After vaccination, a physician monitored subjects for any immediate sign or symptom of local and/or systemic reactions for at least 30 min. We asked the parents or legal guardians to record any adverse reactions occurred from days 0-42 after vaccination. Significant local reaction was defined as redness > 2.5 cm, swelling > 2.5 cm, or tenderness, or crying and protesting when the injection site was touched. Systemic reactions included vomiting, diarrhea, irritability, drowsiness, loss of appetite, shivering, or fever. Fever was defined as a temperature ≥ 38°C (100.4°F) (16). Symptoms were defined as being related if there was a reasonable possibility that the vaccine contributed to the adverse event. Unsolicited local or systemic reactions (with onset date, intensity and resolution) were recorded throughout the 42-day period after each vaccination. Serious adverse events were reported for the duration of the study. Blood pressure, pulse, and body temperature of all subjects were recorded at every visit, and physical examinations were performed.

Statistical analysis
Based on the premise of a 98% production rate of seroprotective antibody, a 10% allowable error for the trial and 10% level of significance, the minimum necessary number of subjects was found to be 65 in this study. Immunogenic response was assessed by the percentage of subjects with seroprotective neutralizing antibody titer and by calculation of the geometric mean titer (GMT) values. For the GMT, 95% confidence intervals (CI)
were calculated using a percentile-based bootstrap method stratified by baseline serostatus. The percentages of subjects who experienced solicited and unsolicited adverse reactions were expressed. Missing data were excluded.

**Ethics statement**
This study was approved by the institutional review board of each study site (IRB number OCMC07MI020 and OCMC08MI033). Written informed consent was obtained from the parents or legal representative of all subjects before study enrollment.

**RESULTS**

**Subjects**
Ninety subjects were enrolled to receive the first dose vaccine at five study centers. The male to female ratio was 1.0, and the median age at enrollment was 12 ± 4 months (87 children age 12-23 months, two 24-35 months, and one 36-47 months). There were no withdrawals due to adverse events after the first vaccination. Among all subjects enrolled at the first vaccination, 19 subjects were lost to follow up at the second vaccination and 2 voluntarily withdrew participation. Overall, a total of 69 subjects completed the following study, thus receiving the second dose. Three samples after the second dose were not included in the immunogenicity analysis for reasons expected to impact immunogenicity such as erroneous sampling or inappropriate sampling time, but these cases were not excluded from the safety analysis. The male to female ratio of the follow-up group was 1.25, and the median age was 24 ± 4 months (67 children age 24-35 months, one 36-47 months, and one 48-59 months).

**Immunogenicity results**
The immunogenicity results are summarized in Table 1. Sero-protection to the first dose was observed in 91.1% (82/90) of subjects. The GMT showed an increase of 3-fold (40.90, 95% CI, 30.09-50.58), from 12.86 (95% CI, 10.67-15.51) at baseline.

At the 1-yr follow-up, 4 out of 66 subjects had no detectable neutralizing antibody. Overall, the seroprotection rate following the second dose rose to 97% (64/66), compared to 93.9% (62/66) prior to vaccination. The GMT was measured from 33.05 (95% CI, 24.42-44.60) to 213.84 (95% CI, 151.54-301.59), representing a 6.47-fold rise.

**Safety**
The results of adverse reactions after each dose are summarized in Table 2. Adverse events reported by all subjects after the first dose were mild to moderate in severity and self-limited in duration, with 18.9% of subjects reporting one or more adverse events. The most frequently reported local reactions after the first dose were redness (13.3%) and tenderness (8.9%) at the injection site. Systemic reactions were infrequent with the exception of fever (4.4%), but no subject experienced a fever ≥ 40°C. During the 42 days following vaccination, unsolicited adverse events were experienced by 30% of children. Most unsolicited adverse events were not related to vaccination, and included nasopharyngitis (6.7%), rhinitis (6.7%), gastroenteritis (6.7%), skin rash (3.3%), and conjunctivitis (2.2%).

The incidence of solicited local reactions was slightly higher after the second dose (30.3%) than the first dose. The most frequently reported local reaction was redness (13.3%) and tenderness (8.9%) at the injection site. Systemic reactions were infrequent with the exception of fever (4.4%), but no subject experienced a fever ≥ 40°C. During the 42 days following vaccination, unsolicited adverse events were experienced by 30% of children. Most unsolicited adverse events were not related to vaccination, and included nasopharyngitis (6.7%), rhinitis (6.7%), gastroenteritis (6.7%), skin rash (3.3%), and conjunctivitis (2.2%).

The incidence of solicited local reactions was slightly higher after the second dose (30.3%) than the first dose. The most frequently reported local reaction was redness and tenderness, which occurred in 24.6% and 20.3% of subjects, respectively. Fever was developed in 7.2% subjects after the second dose. There were no serious adverse events, deaths, or withdrawals from the study due to any type of adverse event.

**DISCUSSION**

Over the past decades, there has been the geographical expansion of JE to new territories, and recurrent outbreaks in Vietnam,
Based on consideration of cost-benefit effect, there is a need for carefully planned studies to establish more optimal immunization schedule.

In this study, LAJEV was safe and well-tolerated. Reactogenicity was satisfactory, although local reactions tended to be more frequent after the second dose. The systemic reactions were mainly fever, which was seen in less than 10% of subjects during the study, and which resolved spontaneously in all cases. Most adverse events were relatively mild and self-limiting. There were no reports of encephalitis or aseptic meningitis following vaccination. In a prospective, randomized study where patients were actively monitored for 30 days, no cases of encephalitis or meningitis were observed, and no differences in hospitalization or prolonged fever were found (10). However, recent encephalitis cases have been reported in Chinese patients who received a first dose of LAJEV, although it is difficult to identify the causal link between the disease and JE vaccination (27). Therefore, concerns about safety still remain, and continued surveillance and careful investigation of any events that occur after LAJEV is required.

Some limitations of our study must be considered. First, the number of study subjects were relatively small. However, the study design was prospective, and the serial assessment of immunogenicity and safety of LAJEV over 1 yr was firstly done in Korean children with local schedule. Second, it is necessary to observe the long-term safety of this vaccine. Although severe neurologic or hypersensitivity reactions did not occur during this study, the prolonged observation of adverse events is required. Third, we did not assess the interchangeability of other available JE vaccines. Because the national immunization program (NIP) in Korea includes inactivated JE vaccine and LAJEV (approved as NIP vaccine in 2014), clinicians can face this situation considering interchangeable vaccination when a patient is lacking a vaccination record or there is a limited vaccine supply from a particular manufacturer. These limitations need to be addressed in future studies.

In conclusion, our findings provide evidence of high protection rate elicited by a 2-dose schedule of LAJEV given 1 yr apart. With respect to vaccine safety, there was no severe vaccine-related adverse events. Although the data is insufficient to conclude whether LAJEV can prevent JE outbreaks, we expect that this immunization strategy will decrease the susceptibility level and increase herd immunity in affected regions, and thereby substantially reduce the burden of JE.

DISCLOSURE

The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTION

Conception and coordination of the study: Kang JH. Design of ethical issues: Lee SY, Kim KH, Kim DS, Cha SH, Jo DS, Kang...
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