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Safety and immunogenicity of live-attenuated Japanese encephalitis SA 14-14-2 vaccine co-administered with measles vaccine in 9-month-old infants in Sri Lanka

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ABSTRACT

Introduction: To facilitate introduction of live attenuated SA 14-14-2 Japanese encephalitis vaccine (LJEV) into the National Immunization Programme of Sri Lanka, we evaluated the safety and immunogenicity of co-administration of LJEV and measles vaccine at 9 months of age. Serum immune responses were evaluated post-vaccination on days 28, 180, and 365 using JE neutralization test and anti-measles IgG ELISA.

Results: 278 infants received one dose of LJEV and measles vaccine. Of these, 257 were eligible for the per-protocol analysis. On Day 0, 14 infants (5.5%) were seropositive for JE, but none were seropositive for measles. At Day 28, seropositivity rates were 90.7% (95% CI, 86.4–93.9%) for JE and 84.8% (95% CI, 79.8–89.0%) for measles. The geometric mean titer for JE neutralizing antibodies was 111 (95% CI, 90–135), and the geometric mean concentration (GMC) for anti-measles IgG was 375 mIU/mL (95% CI, 351–400 mIU/mL). Over the next year, JE neutralizing antibody responses declined only slightly, with seropositivity at 87.4% (95% CI, 82.6–91.2%) at Day 365. In contrast, measles antibody levels continued to increase over time. Seropositivity for anti-measles IgG reached 97.2% (95% CI, 94.4–98.9%) at Day 365, and the GMC rose to 1202 mIU/mL (95% CI, 1077–1341 mIU/mL). Co-administration of LJEV and measles vaccine was also safe. Most adverse reactions were mild, and no serious adverse events were related to study vaccinations.

Conclusion: The safety and immunogenicity of LJEV co-administered with measles vaccine in Sri Lankan infants is similar to that seen in other populations, and our results support use of LJEV at 9 months of age. Live SA 14-14-2 vaccine is now prequalified by the WHO for use in infants in Asia, and other countries may wish to introduce LJEV to combat this devastating disease.

1. Introduction

Japanese encephalitis (JE) virus is an arbovirus that causes a devastating neurological disease resulting in high rates of mortality or neurologic sequelae. The severity of sequelae, together with the volume of cases, makes JE an important cause of encephalitis [1,2]. The disease is endemic across temperate and tropical zones of Asia, and because of its zoonotic cycle, eradicating JE from the environment is unrealistic. Universal childhood vaccination is essential for disease control.

In Sri Lanka, immunization against JE began in 1988. By 2006, two types of JE vaccines were available for use in Sri Lanka—inactivated mouse brain-derived vaccine and live attenuated SA-14-14-2 JE vaccine (LJEV). Only the inactivated vaccine was being used in the country's public-sector immunization program. Concern in Japan over a rare but potentially dangerous adverse event associated with a mouse brain-derived vaccine led

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the manufacturer in Japan to discontinue production in 2005, thus limiting global supply of inactivated JE vaccines and raising costs for remaining inactivated vaccines.

In August of 2006, the World Health Organization stated in its position paper on Japanese encephalitis vaccines that the mouse brain-derived vaccine should be replaced by a new generation of JE vaccines [3]. For Sri Lanka, switching to the less expensive LJEV was estimated in 2006 to save the National Immunization Programme (NIP) between US$8.6 and $8.9 million annually in direct vaccine costs alone. To generate local immunogenicity and safety data to guide policy for potential use of LJEV in Sri Lanka's NIP, the Ministry of Healthcare and Nutrition, in cooperation with PATH, initiated the current study.

2. Methods

2.1. Study design and population

This open label, non-randomized, single-arm trial was designed to evaluate the immunogenicity and safety of the co-administration of LJEV and measles vaccine among infants in order to facilitate introduction of LJEV into the Sri Lankan NIP at 9 months of age. The study was conducted from July 2007 to October 2008 in three peri-urban health divisions of low JE endemicity in the District of Colombo. Healthy infants 9 months of age (plus or minus 2 weeks) who could be adequately followed for safety and who could attend all scheduled study visits were eligible. Infants with a history of measles or Japanese encephalitis (or major symptoms of either disease), or a history of previous receipt of any vaccine against these diseases, were excluded. Non-study vaccinations were restricted to between 2 weeks prior to enrollment until 28 days after study enrollment.

2.2. Study vaccines

At enrollment, all eligible participants were administered one dose of LJEV (SA 14-14-2, Chengdu Institute of Biological Products [CDIBP], Chengdu, China; batch 200611A078-1) subcutaneously in the right brachium and one dose of measles vaccine live, attenuated (Serum Institute of India, Ltd, Pune, India; batch EU3244) subcutaneously in the left brachium.

2.3. Primary and secondary outcomes

2.3.1. Immunogenicity

Blood serum was collected immediately before administration of study vaccines and approximately 28 days and 1 year later. After study initiation, the protocol was amended to request an additional blood specimen at six months post-co-administration from additionally consented participants.

Primary immunogenicity objective outcomes were the proportion of subjects with demonstrated seropositivity for JE and measles at 28 days post-co-administration. Serum neutralizing antibodies to the Bejing-1 JE strain were measured by plaque reduction neutralization test (PRNT) where the neutralizing titer was measured as the inverse dilution at which plaque counts were reduced by 50%. Seropositivity for JE was then defined as a neutralizing antibody titer of $\geq$1:10, as recommended by the WHO [4]. Serum anti-measles immunoglobulin class G (IgG) antibodies were measured by enzyme-linked immunosorbent assay (ELISA) (Serion ELISA *classic* Measles Virus IgG, Serion GmbH, Würzburg, Germany). Seropositivity for measles was defined per the manufacturer's instruction as an antibody concentration of >200 mIU/mL; "borderline" was 150–200 mIU/mL.

Secondary immunogenicity outcomes included the geometric mean titer (GMT) of serum neutralizing antibody to JE and the geometric mean concentration (GMC) of anti-measles IgG at 28 days post-co-administration of study vaccines. Additional secondary objectives were immunogenicity at 6 months post-co-administration and at 1 year post-co-administration. In a separate *post-hoc* analysis, immunogenicity was also analyzed counting as seropositive all infants with "borderline" anti-measles IgG concentrations.

2.4. Safety

All adverse reactions and adverse events were captured from the time of co-administration of study vaccines until 28 days later. Serious adverse events (SAEs)—as defined by ICH GCP and with the additional criterion of "important medical events that may not result in death, be life threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed by ICH GCP"—occurring at any time during the study were further documented.

During the 7 days post-co-administration of study vaccines parents completed diary cards for solicited and unsolicited events; parents were given specific grading scales for solicited events and a generic grading scale to apply to unsolicited events. Study physicians visited the homes of study subjects 2 or 3 days post-vaccination to check that completion of diary cards was proceeding well and to assist parents with any questions or problems. Additional unsolicited events were captured through parental interview and graded by study physicians at the 28-day study clinic visit. After the 28-day study clinic visit, participants were visited or telephoned monthly by trained physicians until the end of the study to identify only SAEs. SAEs were graded for severity using the generic grading scale for unsolicited events.

2.5. Statistical analyses

The study was designed to estimate simultaneously seropositivity for JE and measles antibodies 28 days post-vaccination. The primary analysis of immunogenicity was based on the per-protocol subject population. Seropositivity rates and corresponding exact 95% confidence intervals (CIs) were calculated based on the binomial distributions of study outcomes. GMTs and corresponding 95% confidence intervals were calculated based on the normal distributions. For calculations of JE GMTs, titers less than the limit of detection were assigned a value of 1:5.

We assumed the Day 28 post-co-administration seropositivity would be 90% [5] for JE and 95% [6] for measles. Under these assumptions, a sample size of 249 evaluable subjects was required to demonstrate with at least 80% power that the observed seropositivity rate for JE antibodies is greater than 80% *and* that the observed seropositivity rate for measles antibodies is greater than 90%, using one-sided significance levels of 0.025. We planned to consent up to 312 infants to allow for up to 10% exclusion during screening and 10% loss to follow-up.

2.6. Ethics

At the end of the study, any child who had not successfully seroconverted for JE and/or measles was offered revaccination free of cost. The study was approved by the University Of Colombo Faculty Of Medicine Ethical Review Committee and PATH's Research Ethics Committee, USA. Written informed consent was obtained from parents or guardians of all participants. The study was conducted in accordance with the principles of the Declaration of Helsinki and in compliance with the International Conference on