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Live-attenuated, tetravalent dengue vaccine in children, adolescents and adults in a dengue endemic country: Randomized controlled phase I trial in the Philippines

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ABSTRACT

A recombinant live attenuated tetravalent dengue vaccine (TDV) is safe and immunogenic in adults and children in dengue-naïve populations. Data are needed in dengue endemic populations. In a phase I, randomized, controlled, blind-observer study in the Philippines, groups of participants aged 2–5, 6–11, 12–17, and 18–45 years received either three TDV vaccinations at months 0, 3.5, and 12 (TDV–TDV–TDV group) or licensed typhoid vaccination at month 0 and TDV at months 3.5 and 12 (TyVi–TDV–TDV group) and were followed for safety (including biological safety and vaccine virus viremia) and immunogenicity. No serious adverse vaccine related events and no significant trends in biological safety parameters were reported. Injection site pain, headache, malaise, myalgia, fever, and asthenia were reported most frequently, as mild to moderate in most cases and transient. Reactogenicity did not increase with successive vaccinations and was no higher in children than in adults and adolescents. Low levels of vaccinal viremia were detected in both groups after each TDV vaccination. After three TDV vaccinations, the seropositivity rates against serotypes 1–4 were: 91%, 100%, 96%, 100%, respectively, in 2–5 year-olds; 88%, 96% 96%, 92% in 6–11 year-olds; 88%, 83%, 92%, 96% in adolescents; and 100% for all serotypes in adults. A similar response was observed after two doses for the TyVi–TDV–TDV group. The safety profile of TDV in a flavivirus endemic population was consistent with previous reports from flavivirus naïve populations. A vaccine regimen of either three TDV vaccinations administered over a year or two TDV vaccinations given more than 8 months apart resulted in a balanced antibody response to all four dengue serotypes in this flavivirus-exposed population, including children.

1. Introduction

Dengue disease is caused by four antigenically distinct, but closely related dengue virus serotypes (1–4), of the genus flavivirus [1]. Dengue disease is usually transmitted by the mosquito *Aedes aegypti*. The incubation period of dengue after the mosquito bite averages 4 days (range 3–14 days) and while dengue can be asymptomatic there is a range of dengue illnesses from non-specific viral syndrome to severe, fatal hemorrhagic disease [2,3]. Dengue fever (DF) is characterized by biphasic fever, headache, myalgia, rash, and lymphadenopathy, and dengue hemorrhagic fever (DHF) by homeostasis abnormalities, platelet decrease and increased vascular permeability, which can lead to dengue shock syndrome (DSS). Recovery from DF is usually complete in seven to ten days but residual asthenia can last several weeks. In Asia, DHF and DSS are observed primarily in children, with approximately 90% of those with DHF being less than 15 years of age [4,5]. In contrast, outbreaks in Caribbean and Central America have predominantly affected adults [4].

In the Philippines dengue is endemo-epidemic with almost 58,000 cases and 548 deaths occurring in 2009 [6]. DHF was first identified in the Philippines and Thailand during the 1950s and it now affects most Asian countries. It is becoming the leading cause of hospitalization in children in the region [7]. All four dengue serotypes are present in the Philippines [8].

Dengue prevention measures, such as mosquito control and personal protection from bites, are limited in efficacy, difficult to enforce, and expensive. A safe and effective vaccine directed against the four serotypes of dengue virus responsible for the disease would be the best mode of prevention; however, there is currently no licensed vaccine of this type available.

This primary manuscript reports the findings of a phase I study conducted in the Philippines – a dengue and Japanese encephalitis endemic country – to evaluate the safety and immunogenicity of a

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tetravalent dengue vaccine (TDV), designed to provide protection against dengue infection. The vaccine is composed of 4 monovalent live-attenuated virus vaccines, each expressing the pre-membrane and envelope proteins of one of the four dengue serotype (serotypes 1–4), whose genes have been inserted in place of the corresponding genes of the attenuated yellow fever virus vaccine strain, YF 17D [9,10]. As children in Flavivirus endemic countries represent the primary population for vaccination, it is important to assess the safety and immunogenicity of the vaccine also in these populations.

2. Materials and methods

2.1. Trial design and participants

A total of 126 volunteers were enrolled in a phase I blind-observer (for the first vaccination, and open for the second and third vaccinations) single-center study in the Philippines. Participants were randomized in two groups in a ratio of 2:1 and received either: (i) three vaccinations of TDV at months 0, 3.5, and 12 or (ii) one vaccination of a licensed control typhoid vaccine (Typhim Vi®) at day 0, followed by two TDV vaccinations at months 3.5 and 12. As a safety precaution we used a step-wise approach for this trial and recruited participants from four age cohorts: 18 adults aged 18–45 years were vaccinated first, then 36 adolescents aged 12–17 years, followed by 36 children aged 6–11 years, and finally 36 children aged 2–5 years. An independent data monitoring committee reviewed and evaluated the results from each step in the vaccination process before approving progression to the next step.

Participants were considered ineligible if they had any of the following: a history of thymic pathology, thymectomy or myasthenia; any immunodeficiency or chronic illness that could interfere with the trial conduct or results; previous vaccination against typhoid or flavivirus diseases, or a flavivirus vaccination planned during the current trial. Women who were breast-feeding or pregnant were also excluded and all enrolled women who were of childbearing potential were required to use an effective method of contraception or sexual abstinence for at least four weeks before the first vaccination and at least four weeks after each vaccination. Finally, volunteers with a history of flavivirus infection were to be excluded, but there was no screening for flavivirus-specific antibodies.

This trial was conducted in accordance with the ethical principals set out in the Declaration of Helsinki and Good Clinical Practice, International Conference on Harmonization guidelines, the European Directive 2001/20/EC, and the applicable national and local requirements. All adult participants and the parents or legal guardians of participants under 18 years of age provided their written informed consent. In addition, participants who were 8–11 years of age were asked to provide their written consent using a specific form containing simplified information about the trial (assent form).

2.2. Vaccines

TDV was provided as a frozen liquid in single-dose vials, containing approximately 5 $\log_{10}$ cell culture infectious dose of each of the four live attenuated chimeric dengue vaccine viruses per 0.5 mL dose. TDV was formulated in minimal essential medium containing human serum albumin United States pharmacopoeia (2.5%) and lactose (7.5%).

The licensed typhoid vaccine used as the control for the month 0 vaccination was a liquid vaccine and composed of 25 μg of the *Salmonella typhi* (Ty2 strain) with purified Vi capsular polysaccharide per 0.5 mL dose. The vaccine was formulated in 0.25% phenol as a preservative and an isotonic buffer solution. Both vaccines were manufactured and supplied by Sanofi Pasteur (Lyon, France) and were administered subcutaneously in the deltoid region.

Table 1
Definitions of solicited injection site and systemic reactions.

Reactions	Definitions
Solicited injection site reactions	
Pain	Mild: easily tolerated Moderate: sufficiently discomforting to interfere with normal behavior or activities Severe: incapacitating, unable to perform usual activities, may have/or required medical care or absenteeism
Erythema and swelling	Mild: <2.5 cm Moderate: 2.5–4.9 cm Severe: ≥5 cm
Solicited systemic reactions	
Fever	Mild: 37.4–37.9 °C Moderate: 38.0–38.9 °C Severe: ≥39.0 °C
Headache, malaise, myalgia and asthenia	Mild: noticeable Moderate: interfered with daily activity Severe: prevented daily activity

2.3. Safety

We monitored participants for 30 min following each vaccination and any adverse events (AEs) during this period were recorded as immediate AEs. We provided participants or parents with safety diary cards, digital thermometers and flexible rulers to record solicited injection site and systemic reactions (Table 1), as well as unsolicited AEs, in an identical manner to the two previously reported phase I studies with this dengue vaccine candidate [15,16]. Solicited injection site reactions and solicited systemic reactions were recorded daily for 7 and 14 days after each vaccination, respectively. Body temperature was measured twice daily though to day 14. Unsolicited injection site reactions and systemic adverse events were recorded for 28 days after each vaccination. Serious adverse events were documented and followed-up at any time during the study. Study staff contacted participants and parents on days 1, 3, 5, 9, 11 and 21 after vaccination to follow-up for any adverse events and to ensure that diary cards were being completed.

We reviewed diary card entries with the participants at each visit and transcribed the information to case report forms. By convention, all solicited events were considered as related to vaccination and referred to as reactions. Other adverse events were classed as vaccine-related (and are referred to as reactions) or unrelated. Biochemistry (renal and liver function tests) and hematology (full blood cell count) analyses were performed on samples obtained before (screening) and 7, 14, and 28 days after the first two injections, and before (screening) and 7 and 28 days after the third.

The investigator reviewed laboratory results and reported abnormal values in the case report form with an assessment of clinical significance and potential relationship to the vaccine.

2.4. Viremia

The presence of the four dengue vaccine viruses in serum was detected using quantitative and serotype-specific reverse transcriptase-polymerase chain reaction (RT-PCR). Assessments were carried out on the day of each injection, 7 and 14 days following the first and the second injections, and 7 days following the third injection similarly to that described by Mantel et al. in 2008 [11]. Briefly, a non-serotype-specific quantitative RT-PCR