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Biodistribution and safety of a live attenuated tetravalent dengue vaccine in the cynomolgus monkey

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ABSTRACT

Background: The first licensed dengue vaccine is a recombinant, live, attenuated, tetravalent dengue virus vaccine (CYD-TDV; Sanofi Pasteur). This study assessed the biodistribution, shedding, and toxicity of CYD-TDV in a non-human primate model as part of the nonclinical safety assessment program for the vaccine.

Methods: Cynomolgus monkeys were given one subcutaneous injection of either one human dose (5 $\log_{10}$ $CCID_{50}$/serotype) of CYD-TDV or saline control. Study endpoints included clinical observations, body temperature, body weight, food consumption, clinical pathology, immunogenicity, and post-mortem examinations including histopathology. Viral load, distribution, persistence, and shedding in tissues and body fluids were evaluated by quantitative reverse transcriptase polymerase chain reaction.

Results: The subcutaneous administration of CYD-TDV was well tolerated. There were no toxicological findings other than expected minor local reactions at the injection site. A transient low level of CYD-TDV viral RNA was detected in blood and the viral genome was identified primarily at the injection site and in the draining lymph nodes following immunization.

Conclusions: These results, together with other data from repeat-dose toxicity and neurovirulence studies, confirm the absence of toxicological concern with CYD-TDV and corroborate clinical study observations.

1. Introduction

Dengue virus (DENV) is a member of the *Flavivirus* genus, principally transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes [1]. Clinical manifestations following infection by one of the four DENV serotypes (DENV-1–4) range from asymptomatic, to a self-limiting acute febrile illness (dengue fever [DF]), and through to potentially life-threatening syndromes; dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2]. The disease is endemic in more than 100 countries, with recent estimates suggesting a dramatic increase in the global burden over the last decades [3]. Dengue prevention has until now been primarily through vector control [2], and the development of preventive vaccines is a priority. The first licensed dengue vaccine, a live, attenuated, tetravalent dengue vaccine (CYD-TDV; Dengvaxia®), has recently been registered in 15 countries as a 3-dose immunization schedule administered subcutaneously at 6-month intervals [4].

CYD-TDV is composed of live attenuated genetically engineered chimeric viruses, constructed by replacing the genes encoding for the pre-membrane and envelope structural proteins of the attenuated yellow fever (YF) 17D vaccine virus genome by the corresponding genes of the four wild-type (wt) dengue serotypes. A nonclinical safety program was undertaken to support the clinical development and registration of CYD-TDV. While humans and mosquitoes are the only natural hosts for DENV infections, the

Abbreviations: CYD-TDV, live attenuated tetravalent dengue vaccine; DENV, dengue virus; DF, dengue fever; DHF, dengue hemorrhagic fever; DSS, dengue shock syndrome; ELISA, enzyme-linked immunosorbent assay; LLOQ, lower limit of quantification; NHPs, non-human primates; PBS, phosphate buffered saline; $PRNT_{50}$, plaque reduction neutralization test 50; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; sc, subcutaneous; WHO, World Health Organization; wt, wild-type; YF, yellow fever; YF-NS5, yellow fever NS5 gene.

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virus replicates in non-human primates (NHPs) but does not induce the development of dengue disease [5]. NHPs are recommended by the World Health Organization (WHO) as suitable preclinical models for the evaluation of dengue vaccine candidates under development [6], and demonstrate both a measurable immune response with neutralizing antibodies to the vaccine and a consistently measurable viremia [7]. NHPs are also an established model for general toxicity assessment of vaccines [8] and were selected as the animal model for preclinical safety development of CYD-TDV.

As part of the nonclinical safety program, the biodistribution, shedding and toxicity of CYD-TDV after a single dose were evaluated in NHPs. Safety endpoints were correlated to any replication and/or persistence in the tissues to address the potential risk of viscerotropism and neurotropism, which could be linked to the inherent viscerotropic and/or neurotropic CYD parental viruses [1,9].

2. Materials and methods

2.1. Animals and housing

Forty-two naïve purpose-bred cynomolgus monkeys (*Macaca fascicularis*; 21 males and 21 females) aged 27–29 months were purchased from Le Tamarinier (Mauritius). After 40-day quarantine and 10-day acclimation periods, the animals were group-housed in stainless steel housing units under controlled conditions (19–25 °C, 50 ± 30% humidity, approximately 12 cycles per hour of filtered, non-recycled air, 12-h light cycle) with free access to tap water and daily distribution of expanded diet (SDS, France) and fruits. When necessary, animals were housed in individual cages to allow blood, urine and feces collection or individual follow-up. Blood samples were collected during the 10-day acclimation period, and analyzed for antibodies against the flaviviridae viruses by enzyme-linked immunosorbent assay (ELISA). Only flavivirus-seronegative animals were used for the study and selected based on clinical examinations, body weights and clinical pathology data. Animals were allocated to two study groups by gender using a manual randomization procedure.

2.2. Vaccination

On day 1, the monkeys were given a single subcutaneous (sc) injection of CYD-TDV (15 animals of each sex) or control (0.9% sodium chloride, Lavoisier, France; 6 animals of each sex) in the deltoid region. The animals were given CYD-TDV manufactured under Good Manufacturing Practices from the lot used in two phase III clinical trials [10,11]. CYD-TDV was presented as a lyophilized powder reconstituted in 0.5 mL saline (0.4%) solution; each dose contained approximately 5 ± 1 $\log_{10}$ $CCID_{50}$ of each of the four live, attenuated, recombinant CYD serotypes. The dose and volume were identical to those used in the pivotal trials.

2.3. In-life observations

The distribution, persistence/elimination and safety of CYD-TDV were evaluated in three sets of monkeys (5 vaccinated and 2 control animals of each sex) over 3-, 9- and 21-day observation periods, respectively. The 3- and 9-day observation periods were selected to cover the period of expected viremia, based on previous studies [7,12]. All monkeys were observed daily for mortality, adverse clinical signs, local injection site reactions and body temperature (rectal). Body weight was recorded pre-dose, on the day of treatment and then weekly until necropsy. Blood samples for hematology, coagulation, and biochemistry and urine samples were collected pre-dose and on days 4, 10 and 22 corresponding to the end of each observation period for the three sets of monkeys.

Hematology parameters, including erythrocyte count, hemoglobin concentration, mean and packed cell volumes, mean cell hemoglobin concentration, mean cell hemoglobin content, thrombocytes, white blood cells including differential and reticulocyte counts were analyzed by ADVIA 120 (Siemens, France) using samples collected into EDTA tubes. Coagulation parameters, including prothrombin time, activated partial thromboplastin time and fibrinogen were analyzed by ACL Elite Pro (Beckman Coulter, Instrumentation Laboratory, France), using samples collected into sodium citrate tubes. Blood biochemistry parameters, including sodium, potassium, chloride, calcium, inorganic phosphorus, glucose, urea, creatinine, total bilirubin, total proteins, albumin, globulin, albumin/globulin ratio, total cholesterol, triglycerides, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase were analyzed by ADVIA 1650 (Siemens, France) using samples collected into lithium heparin tubes.

Urinalysis including volume, pH, specific gravity, proteins, creatinine, glucose, ketones, bilirubin, nitrites, blood (hemoglobin) and urobilinogen was conducted with Clinitek 500 or ADVIA 1650 (Siemens, France). Cytology of sediments, including leucocytes, erythrocytes, cylinders, magnesium ammonium phosphate crystals, calcium phosphate crystals, calcium oxalate crystals and epithelial cells was analyzed by microscopic examination.

2.4. Viral load and shedding

Samples for viral load in blood and viral shedding in body fluids (urine, feces, saliva swab and injection site swab) were taken pre-dose, daily over days 1–12 and at necropsy. The blood and urine samples were centrifuged (800*g* for 10 minutes at +4 °C) and supernatant was retained for analysis. Feces samples were diluted (1/5 w/v) with cold phosphate buffered saline (PBS), centrifuged under similar conditions, supernatant was filtered and retained for analysis. The swabs for saliva and skin at the injection site were collected into tubes with PBS. All samples were snap frozen in liquid nitrogen and stored at −80 °C pending virus quantification by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).

2.5. Necropsies and tissue sampling

At the end of the observation/assessment periods each group of animals were deeply anesthetized and euthanized with an intramuscular injection of ketamine hydrochloride/xylazine. Each animal received an intracardiac infusion of cold sterile isotonic saline solution (+4 °C, for 10 min, 150 mL/min). The list of organs sampled and type of assessments undertaken is summarized in Table 1. For qRT-PCR analysis, tissues were rinsed with cold PBS at +4 °C. Cerebrospinal fluid was collected from each animal into cryotubes containing heat inactivated fetal bovine serum. Urine was directly collected from the bladder and feces from the colon. Selected tissues were processed for quantification of viral gene by qRT-PCR and for histopathological examination (Table 1). All samples for distribution or shedding analysis were immediately snap frozen in liquid nitrogen and stored at −80 °C until analysis. Selected tissues for histopathology were fixed in formalin and embedded in paraffin wax; 4 μm sections were prepared and stained with hematoxylin-eosin prior to examination.

2.6. Quantitative reverse transcriptase-polymerase chain reaction

CYD virus RNA levels in tissues and body fluids were initially determined through identification of non-serotype specific vaccine RNA, Yellow Fever NS5 gene (YF-NS5), on extracted RNA using a

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