Vaccine 32 (2014) 1055–1060

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Evaluation of immune responses to an oral typhoid vaccine, Ty21a, in children from 2 to 5 years of age in Bangladesh

Taufiqur R. Bhuiyan[a], Feroza K. Choudhury[a], Farhana Khanam[a], Amit Saha[a], Md. Abu Sayeed[a], Umme Salma[a], Anna Lundgren[b], David A. Sack[c], Ann-Mari Svennerholm[b], Firdausi Qadri[a,*]

[a] Centre for Vaccine Sciences, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh
[b] Department of Microbiology and Immunology, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden
[c] Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

ARTICLE INFO

Article history:
Received 15 July 2013
Received in revised form
19 December 2013
Accepted 2 January 2014
Available online 15 January 2014

Keywords:
Typhoid vaccine
Oral
Children
Antibodies
Mucosal
T cells

ABSTRACT

Young children are very susceptible to typhoid fever, emphasizing the need for vaccination in under five age groups. The parenteral Vi polysaccharide vaccine is not immunogenic in children under 2 years and the oral Ty21a vaccine (Vivotif) available in capsular formulation is only recommended for those over 5 years.

We studied immune responses to a liquid formulation of Ty21a in children 2–5 years of age. Since children in developing countries are in general hypo responsive to oral vaccines, the study was designed to determine if anti-helminthic treatment prior to vaccination, improves responses.

In a pilot study in 20 children aged 4–5 years, the immune responses in plasma and in antibody in lymphocyte secretions (ALS) to the enteric coated capsule formulation of Ty21a was found to be comparable to a liquid formulation ($P > 0.05$). Based on this, children ($n = 252$) aged ≥2–<3 years and ≥3–<5 years were randomized to receive a liquid formulation of Ty21a with and without previous anti-helminthic treatment.

The vaccine was well tolerated with only a few mild adverse events recorded in <1% of the children. Deworming did not improve immune responses and both age groups developed 32–71% IgA, IgG, and IgM responses in plasma and 63–86% IgA responses in ALS and stool specimens to a membrane preparation (MP) of Ty21a. An early MP specific proliferative T cell response was also seen.

We recommend that safety and efficacy studies with a liquid formulation of the vaccine are carried out in children under five, including those less than two years of age to determine if Ty21a is protective in these age groups and applicable as a public health tool for controlling typhoid fever in high prevalence areas of typhoid fever including Bangladesh.

1. Introduction

Typhoid fever is a major problem in developing countries with infants and children as well as adults being affected [1–3]. Along with improvements in water and sanitation, vaccines could play an important role in the control of the disease. However, the two currently available licensed typhoid vaccines, i.e. the parenteral Vi polysaccharide and the oral Ty21a live attenuated vaccine, Vivotif, are not recommended for use in young children below 2 and 5 years of age, respectively. The parenteral Vi polysaccharide vaccine is poorly immunogenic in children under two years [4] and was not protective in a recent study in Pakistan in children 2–5 years [5]. Hence, Vi-conjugate vaccines are being developed and have provided strong protection in young children [6,7], but they are not yet licensed [6–9].

The oral typhoid vaccine, Ty21a, is recommended for children over 5 years and is currently formulated as an enteric coated capsule which young children are not able to swallow. Three doses of vaccine, when given to school age children on alternate days, resulted in significantly decreased incidence of typhoid fever over a 7-year surveillance period [10]. There are only a few studies showing serum antibody responses to Ty21a in children younger than five years. For example, Cryz et al. have reported significant anti-LPS antibody responses in serum of children 2–6 years of age [11]. However, studies of mucosal immune responses against Ty21a in children are still lacking.

Oral vaccines are frequently less immunogenic when given to children in developing countries than when given to adults in developed countries [12]. One hypothesis concerning the lowered

* Corresponding author. Tel.: +880 2 9827001 10x2431/+880 2 9860626; fax: +880 2 8823116.
E-mail address: fqadri@icddrb.org (F. Qadri).

0264-410X/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.vaccine.2014.01.001

Table 1
Demographic characteristics of study participants.

Characteristics	≥2–<3 year old children (*n* = 131)		≥3–<5 year old children (*n* = 121)	
	Treated group (A)[a]	Not treated group (B)[b]	Treated group (A)[a]	Not treated group (B)[b]
Male, no./total (%)	31/65 (48)	34/66 (52)	32/61 (52)	40/60 (67)
Age, median (range), months	34 (24–36)	34 (24–36)	54 (45–58)	54 (40–58)
Weight, median (range), kg	11 (7–17)	12 (8–16)	14 (10–22)	14 (11–24)
Height, median (range), cm	85 (66–105)	87 (71–107)	100 (83–120)	100 (84–122)

[a] Treated: received anti-helminth drugs and three doses of Ty21a vaccine.
[b] Not treated: received placebo and three doses of Ty21a vaccine.

immune responses is that enteric parasites may impair the intestinal immune response. In Bangladesh, *Ascaris lumbricoides* is the most common parasite and as many as 78% of children have been shown to be infested in some studies [13,14]. Other commonly seen parasites include *Trichuris trichuria*, hookworm, and *Giardia lamblia*. In a study conducted in Mirpur, Dhaka, Bangladesh, around 47% of 11–16 years old children were shown to be infected with either *A. lumbricoides* and/or *T. trichiura* [15]. The concern about helminth interfering with oral vaccine responses relates to the finding that infestation with parasites may alter the Th1/Th2 profile of immune responses [16] and one study showed that intestinal parasites impaired vaccine immune responses to cholera vaccine [17].

With the intent to explore the potential usefulness of Ty21a as a suitable vaccine for young children, this study was designed to study the safety and immunogenicity of three doses of Ty21a in children 2–5 years of age and to determine the potential benefit of de-worming to improve immune responses in children with intestinal parasites. In an initial pilot study, we showed comparable immune responses to the licensed capsule and a liquid formulation of Ty21a in a small group of children 4–5 years of age ($n = 20$). Based on these results, the liquid formulation of Ty21a was given to a larger group of children (all subjects were carrying parasites), 2–5 years of age ($n = 252$), to determine mucosal and systemic B and T cell immune responses to the vaccine. We also studied whether treatment with antiparasitic agents (albendazole and secnidazole) could improve the immunogenicity of the vaccine. Although Ty21a has an excellent safety profile, we monitored the subjects for evidence of symptoms following immunization, because this study involved a younger age group for which the vaccine is not approved.

2. Materials and methods

2.1. Study participants, anti-parasitic treatment and vaccine formulation

The study participants were enrolled from a densely populated urban area of Mirpur, Dhaka, Bangladesh between January and August 2011. In an initial pilot study, 20 healthy children 4–5 years of age were randomized to receive three doses of Ty21a ($\geq 2 \times 10^9$ viable *Salmonella* Typhi Ty21a bacteria per dose Ty21a, Crucell, Leiden, The Netherlands) as either a capsule ($n = 10$) or as a liquid formulation ($n = 10$) on days 0, 2 and 4. For those taking the liquid form, children first drank 20 ml of bicarbonate buffer; thereafter the contents of the Ty21a capsule were suspended in 20 ml of normal saline, and this suspension was given to the children 5 min after taking the buffer. For those taking the capsule, children first drank 20 ml of water and 5 min later swallowed the capsule with 20 ml of water.

For the extended study, healthy children 2–5 years of age were screened for the presence of *A. lumbricoides and/or T. trichiura* (Table 1). Among 423 screened children, 252 children who had a parasitic load of ≥100 eggs per gram of stool and whose parents consented were enrolled. We excluded children who had a history of chronic gastrointestinal disorders, diarrheal diseases in the past two weeks, febrile illness in the preceding week, or history of receiving antibiotic treatment within 7 days.

The children enrolled were randomly divided into two equally sized groups; one group (A) was given single dose of a combination of Albendazole (400 mg, Incepta Pharmaceuticals Ltd.) and Secnidazole (500 mg, Incepta Pharmaceuticals Ltd.) and the other group (B) was given placebo in a double blind manner one week prior to vaccination. Those who received placebo prior to vaccine were given the anti-parasitic treatment within 6 months after the study was completed. The analyses were performed separately for children ≥2–<3 years of age (younger children; from second birthday to the day before the fourth birthday) and children ≥3–<5 years of age (older children; from the fourth birthday to the day before the sixth birthday) in each study group. All children in the extended study received three doses of liquid vaccine on days 0, 2 and 4 with buffer as described above 7 days after receiving anti-parasitic drugs or placebo. This study was registered with the Clinical Trials Data Bank (http://clinicaltrials.gov/), Identifier: NCT01019083.

2.2. Specimen collection and preparation

From all children, venous blood specimens (3 ml) were collected immediately before the first immunization (day 0) and then 7 and 21 days after the third dose of vaccination. In addition, stool specimens (ca 2–3 g) were collected on days 0, 7 and 21. Stool specimens were frozen at −80 °C until extracted as previously described [18] and extracts were stored at −80 °C until analyzed.

Peripheral blood mononuclear cells (PBMCs) and plasma were isolated from venous blood by density gradient centrifugation on Ficoll–Isopaque (Pharmacia, Uppsala, Sweden). Plasma collected from the top of the Ficoll gradient was stored in aliquots at −20 °C until analyzed in ELISA tests; after thawing, plasma specimens were centrifuged at 7500 g for 10 min immediately before ELISA analyses.

2.3. ALS assay and analysis of antibody responses in ALS and plasma specimens and stool extracts

PBMCs were suspended in RPMI complete medium RPMI 1640 (Gibco, Gaithersburg, MD) (see the Supplemental materials) and were incubated for 48 h at 37 °C in 5% CO_2 without stimulation for the ALS (antibodies in lymphocyte supernatant) assay [19].

ELISA was used to determine IgA, IgG and IgM responses against Ty21a membrane preparation (MP) in plasma and IgA responses in ALS and stool extract specimens [18,20,21] (the Supplemental materials).

2.4. Bacterial antigens

The vaccine strain Ty21a cultured on horse blood agar plates was used for preparation of a membrane preparation (MP) by sonication followed by differential centrifugation [22–24] (see the Supplemental materials).