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

Taufiqr 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

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 hyporesponsive 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. De-worming 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.

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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
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 infected in some studies [13,14]. Other commonly seen parasites include Trichuris trichiura, hookworm, and Giardia lambila. 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 (≥2 × 10⁸ 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% CO2 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).
Table 2
Geometric mean titers (range) of antibody responses in plasma and ALS* specimens in children vaccinated with the capsular or liquid formulation of Ty21a.

<table>
<thead>
<tr>
<th>Parameters (Titer)</th>
<th>Day 0</th>
<th></th>
<th>Day 7</th>
<th></th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Capsule (n = 10)</td>
<td>Liquid formulation (n = 10)</td>
<td>Capsule (n = 10)</td>
<td>Liquid formulation (n = 10)</td>
<td>Capsule (n = 10)</td>
</tr>
<tr>
<td>Plasma IgA</td>
<td>46 (18–257)</td>
<td>63 (26–237)</td>
<td>87 (12–371)</td>
<td>153 (36–1117)</td>
<td>114 (44–271)</td>
</tr>
<tr>
<td>Plasma IgG</td>
<td>1181 (449–3629)</td>
<td>757 (262–3342)</td>
<td>1496 (652–2996)</td>
<td>1400 (612–2790)</td>
<td>2203 (1245–3663)</td>
</tr>
<tr>
<td>ALS IgA</td>
<td>0.0</td>
<td>0.0</td>
<td>3</td>
<td>13</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* ALS: Antibodies in lymphocyte supernatant, day 0 indicates before vaccination, day 7 and day 21 indicates 7 and 21 days after intake of the 3rd dose of vaccine.

2.5. T cell stimulation assay

In a randomly selected subset of volunteers (n = 30/group), cellular immune responses were analyzed [25] (see the Supplemental materials).

2.6. Statistical analyses

Analysis of data and preparation of graphs were carried out using the statistical software Graphpad prism (Version 5.0) (see the Supplemental materials).

2.7. Ethical approval

The study was approved by the Research Review Committee (RRC) and the Ethical Review Committee (ERC) of icddr,b. Before enrollment in the study, written informed consent was taken from the parents or guardians of the study participants.

3. Results

3.1. Study participants

For the initial comparison of immune responses to the capsule and liquid formulations of Ty21a, we randomized 20 children, 4–5 years of age.

For the evaluation of anti-parasitic treatment prior to vaccination, we randomized 252 children 2 to 5 years of age in two groups (A and B) in a double blinded placebo controlled study; 131 of the children were ≥2–<3 years of age and 121 were ≥3–<5 years. In group A, the participants were treated with the anti-parasitic drugs while group B was given placebo (Table 1). The demography of the participants among anti-parasite treated volunteers and placebo recipients were similar for male/female ratio (P=0.2) and anthropometric measurements including weight, and height indicators.

3.2. Adverse events in study participants after vaccination

All 252 participants aged between 2–5 years age group received Ty21a vaccine. Half of them got anti-helmhinth drugs and half of them got placebo 7 days before vaccination. Adverse reactions were reported in only three participants and not to be related to the intake of the anti-helmhinth treatment or to the vaccine. All of them were in the anti-helmhinth treated children; two of them were in the ≥2–<3 years age group (fever: 2) and one in the ≥3–<5 years age group (respiratory infection: 1).

3.3. Comparison of immune responses in children (≥3–<5 years of age) given capsule and liquid formulation of vaccine

In an initial pilot study in children ≥3–<5 years of age, immune responses generated after intake of the capsular and the liquid formulation of vaccine, respectively were compared (10 children per group). We observed that the vaccine was immunogenic, giving rise to antibody responses to the MP antigen in ALS and plasma specimens. No adverse events were observed in any of the children and both formulations induced mucosal and serum antibody responses. Though slightly higher antibody responses in plasma and ALS specimens at day 7 were observed but no significant differences in IgA, IgG and IgM antibody responses in plasma or IgA responses in ALS specimens to Ty21a-MP antigen were observed after intake of the liquid and capsular formulations (Table 2). These results thus showed that the liquid formulation could be used for studies of the Ty21a vaccine in children who have not reached their 6th birthday.

3.4. Plasma antibody responses to Ty21a-MP in children immunized with a liquid formulation of Ty21a

Antibody responses were determined in children given three oral doses of the liquid formulation of Ty21a. Responses in the different age groups, ≥2–<3 years and ≥3–<5 years, as well as in children with or without anti-parasitic treatment were compared. The responses were measured in plasma, ALS and stool specimens to the MP antigen at 7 and 21 days after vaccination. No significant differences were observed between the anti-parasitic treated (group A) and the un-treated group (group B) (data not shown) for any of the systemic or mucosal immune responses determined. We therefore combined the immune response data for all the children (treated and un-treated) in the respective age groups, ≥2–<3 (n = 131) and ≥3–<5 (n = 121) years of age (Fig. 1). In both age groups, significantly increased MP specific IgA antibody responses in plasma were observed on day 7 as well as on day 21 following vaccination, compared to the pre-immunization level at day 0; 64% (cumulative responder frequencies, CRF) of the children in the ≥2–<3 year age group and 71% (CRF) of the children in the ≥3–<5 years age group responded with a ≥2-fold increase in plasma IgA titers after vaccination. Significant increases of Ty21a-MP specific IgA and IgM antibody responses were also seen in both age groups, predominantly on day 21 but also on day 7. For the IgM isotype, responder frequencies were 39% (CRF) and 32% (CRF) in the ≥2–<3 years and ≥3–<5 years age groups, respectively (Fig. 1); corresponding responder frequencies for the IgG isotype were 45% (CRF) and 47% (CRF). There were no significant differences in magnitude of responses or cumulative responder frequencies for any isotype between the two age groups (Fig. 1).

3.5. Antibody responses in ALS specimens to liquid formulation of Ty21a

Analyses of IgA antibody responses to Ty21a MP in ALS specimens from (both anti-parasite treated and non-treated) children revealed elevated responses on day 7 compared to day 0 in both age groups (Fig. 2). Although antibody levels were lower on day 21 than day 7, responses were also significantly higher on day 21 compared to day 0 in both age groups. 86% of the ≥2–<3 years old and 84% of the ≥3–<5 years old children developed ≥2-fold MP-IgA responses in ALS specimens after vaccination. Comparable antibody response
of years, Ty21a supernatants 3.6. to magnitudes and responder frequencies were observed in the two groups of children \(P = 0.20–0.36\).

3.6. Antibody response in stool extracts to liquid formulation of Ty21a

Mucosal IgA antibody responses against Ty21a MP were also measured in stool extracts in the two age groups. The highest antibody responses \((\geq 2\text{-fold MP-IgA})\) were recorded in the day 7 specimens from most of the children and these responses were significantly higher than the levels detected in day 0 samples. The cumulative responder frequency was 63\% in the \(\geq 2\text{-}3\) years age group and 65\% in the \(3\text{-}5\) years age group (Fig. 3). There were no significant differences in the magnitudes of MP specific IgA antibody response between the two age groups.

3.7. T cell responses to liquid formulation of Ty21a

T cell responses to MP were measured in PBMCs isolated before and 7 and 21 days after immunization in subsets of children in both the \(\geq 2\text{-}3\) years \((n = 30)\) and \(3\text{-}5\) years \((n = 30)\) age groups. No significant differences were observed in either T cell proliferation or cytokine production in the anti-parasitic treated \((n = 15/\text{age group})\) and not treated groups \((n = 15/\text{age group})\) (data not shown) and T cell response data from the two groups were therefore combined.

There was a significant increase in T cell proliferation to MP antigen stimulation from day 0 to day 7 in the younger children and the proliferation increased further on day 21 (Fig. 4). A similar pattern was seen in the older children \((\geq 3\text{-}5\) years). Fifty to sixty percent of the children responded to the vaccination with at least 2-fold increased proliferation in response to MP.

The concentrations of IFN-\(\gamma\), IL-13 and IL-17 were measured in culture supernatants collected after 5 days of stimulation with MP. Stimulated cultures contained relatively high levels of IFN-\(\gamma\), but there were no significant differences in IFN-\(\gamma\) levels in culture supernatants from cells isolated before and after immunization (data not shown). Levels of IL-13 and IL-17 were low or below the detection limit (1 pg/ml) and comparable before and after immunization (data not shown).

4. Discussion

We observed in this study that there was no difference in plasma and ALS antibody titers between anti-helminth treated and untreated young children. However, it has been found that Ty21a was immunogenic in these young children. When testing the Ty21a vaccine in children 2–5 years of age, it was found to be safe. No serious or severe adverse events were observed during the study.
period and only a few non-serious mild events were reported which appeared not to be related to anti-helmint treatment or to vaccine intake in the children that were studied. These results support studied of the Ty21a vaccine in Phase III studies to determine safety, immunogenicity and efficacy in young children between 2–5 years of age in Bangladesh.

In this study, we found that taking the vaccine out of the capsules and suspending it in bicarbonate buffer was a simple approach of preparing a vaccine formulation that could be administered to young children who would have problems ingesting capsules. After having demonstrated similar levels of antibody responses in plasma and ALS specimens after administration of the capsular and liquid formulations of Ty21a in a small pilot study in these young children we used the liquid formulation for the continued studies.

In the larger study of Ty21a vaccine in two age groups of children in >2–<3 and ≥3–<5 years, antibody responses against Ty21a MP in plasma, ALS and stool specimens were analyzed by ELISA. In addition, T cell proliferation and cytokine responses were measured after stimulation of cells with a Ty21a MP antigen. This antigen preparation contains an array of different S. Typhi membrane proteins and also lipopolysaccharide [23–25]. Our MP may contain outer membrane proteins related to those of other gram-negative bacteria. On the other hand, in earlier studies we have shown in MP ELISA, which has been extensively used in our laboratories, seems to be specific and more immunogenic in compare to S. Typhi LPS for the detection of immune responses against natural S. Typhi infection [24] as well as oral typhoid vaccination [20].

Three oral administrations of the liquid preparation of Ty21a, carried out according to the prescribed dosing interval, was shown to induce significant plasma IgA, IgM and IgG antibody responses to Ty21a MP both in children ≥2–<3 years old and in those ≥3–<5 years of age. When analyzing ALS specimens, we observed a significant increase in Ty21a MP specific IgA response after vaccination on day 7 with a decrease on day 21 in a majority of the children in each age group. Similarly, we also found significant immune responses in most of the vaccinated children in both age groups when analyzing their stool extracts. These results indicate that the Ty21a vaccine is capable of inducing substantial mucosal antibody responses in relatively young children living in developing countries. The level of plasma IgG in ≥3–<5 years age group of children increased significantly at day 7, whereas it was not in ≥2–<3 years old age group. It is possible that the higher response in the older children in day 7 is because of prior priming and pre-exposure prior to vaccination that is due to a secondary response to S. Typhi.

Antibody responses in plasma and ALS specimens were measured at day 7 and 21 after the 3rd dose (eg. 11 days after the first dose) of vaccination. Analysis of kinetics of antibody responses in ALS specimens has not been done in this study. Several previous studies have measured ALS and T cell responses to Ty21a 7 days after first vaccination [20]. As a result, we may have missed the peak responses. Higher responses have been shown recently also on day 4 than day 7 after oral cholera booster vaccination [26]. We will investigate the detailed kinetics of immune responses to the Ty21a vaccine in children in a future study.

Our results of immune responses to Ty21a in the children are in agreement with results from other studies of immune responses to Ty21a MP antigen in adults in Sweden [20] vaccinated with the capsular formulation of Ty21a, the later studies have shown significant rises in MP specific IgA and IgG antibody responses in serum as well as IgA antibody secreting cell responses. Furthermore, seroconversion to S. Typhi LPS has been observed in 2–6 years old Thai children after intake of the Ty21a liquid formulation of vaccine [11] which is similar to our present findings.

Previous studies in adults have shown that immune responses to Ty21a are complex, involving not only production of antibodies, but also cell mediated immunity. Antibodies are likely to prevent infection and limit spread of the bacterium in the body, while cell mediated responses may be important for elimination of typhoid bacteria in their intracellular niche and for support of antibody production [27]. In this study, we show that the vaccine induced significant T cell responses in both the younger and the older children, as reflected by increased proliferation in PBMCs stimulated with Ty21a MP after compared to before immunization. These results are consistent with previous studies in adults, showing increased proliferative responses in both PBMCs and purified CD4+ and CD8+ T cells after Ty21a immunization [20,28]. However, in contrast to the strong IFN-γ responses observed in response to vaccination in Swedish adults, IFN-γ responses were relatively weak in most children and not significantly higher than the pre-vaccination levels on a group basis. Weak IFN-γ responses have also been observed to other vaccines in children, potentially as a consequence of low IL-12 production in antigen presenting cells during early life [29,30]. Further studies are needed to elucidate the effector functions of the cell mediated responses in the children, including the cytokine profile of the specific T cells.

In previous studies, it has been shown that anti-helmint treatment may result in improved immune responses against vaccines in developing countries. However, we did not observe any significant differences in typhoid antigen specific antibody levels in plasma, ALS or stool samples or in T cell responses to typhoid antigens between children treated with anti-helmint drugs and those that were not. The lack of influence of anti-helmint treatment on immune responses to the typhoid vaccine may be due to a number of reasons. Most importantly, children in Bangladesh are now routinely given the anti-helmint drugs albendazole at six month intervals in a national de-worming program. We observed the success of this program when we were screening stools for enrolling children and in so many children the highest concentration was around 100 eggs/gm stool. Analyzing the stools we became aware of the impact of this treatment and understand that the level of infection by helmint in stools of children we analyzed is low. In this study design, we included pre-selection for infection for A. lumbricoides or T. trichiura. It would be helpful to better understand about anti-helmint treatment over oral typhoid vaccination in the younger age group of children by incorporating another group of children in whom no parasites are detected. Therefore, the level of infestation in the study children was responsible for observed lack of effect. Hence the low helmint infectious burden may not have affected immune responses to the vaccine. Earlier reports of a positive impact of de-worming on the antibody responses to the
live attenuated cholera vaccine, CVD103HgR in Ecuador was possibly seen since the worm burden was higher in the study group, i.e. around 1000 eggs/stool [17]. In addition, we should follow up the children on the effectiveness of the parasite treatment. One week period may not enough follow up time after anti-helminth treatment for mounting immune responses to oral vaccine. However, we only had IRB permission for the present design. In future studies, we are planning to incorporate longer follow up period.

We did not observe any age dependent differences in antibody responses to the vaccine; the cumulative responder frequency in IgA antibody responses in the two age groups, being 64% and 71%, respectively in plasma and 86% and 84%, respectively in ALS specimens. Hence our results are very promising and suggest that the oral typhoid vaccine can be administered to children 2 years old and above as it does not induce any adverse effects and induces both humoral and cellular immune responses.

In conclusion, our study shows that the oral typhoid Ty21a vaccine is safe and immunogenic in children 2–5 years of age when given in a liquid formulation. Since more than one-fourth (27%) of typhoid cases are shown to occur in children under 2 years of age and in infants [3,31,32], we suggest that a liquid formulation of Ty21a should be tested in younger age groups as well. If Ty21a vaccine is safe and immunogenic also in such young children, we recommend that 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 different countries with a high prevalence of typhoid fever including Bangladesh.

Author Contributions

Conceived and designed the experiments: FQ AMS DAS AL TRB. Performed the experiments: FK FK MAS US. Enrollment of participants: AS FK. Analyzed the data: TRB MAS. Contributed reagents/materials/analysis tools: FQ AMS TRB. Wrote the paper: TRB FK AMS AL DAS FQ.

Conflicts of interest statement

There were no conflicts of interest of any of the authors.

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