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Peru-15, a live attenuated oral cholera vaccine, is safe and immunogenic in Bangladeshi toddlers and infants

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Abstract

A live oral *Vibrio cholerae* O1 El Tor vaccine, Peru-15 was tested in a double-blind, randomized placebo controlled study for safety and immunogenicity in Phase I and Phase II studies in 240 Bangladeshi children aged 9 months–5 years of age. Two different doses (2×10^7 and 2×10^8 cfu) were tested. Vaccination did not elicit adverse events and the strain was genetically stable. Vibriocidal antibody responses developed in 42/50 (84%) toddlers (2–5 years) and 35/50 (70%) of younger children (9–23 months) and overall 77/100 (77%) who received the high dose. LPS-IgA-antibody responses were seen in 60% of toddlers and 34% of infants; 40% responded with IgA antibodies to cholera toxin. The responses to the reduced dose was lower. These studies demonstrate that Peru-15 at a dose of 2×10^8 cfu is safe and immunogenic in children in Bangladesh.

Keywords: Peru-15; Immunogenicity; Bangladeshi children

1. Introduction

A single dose, easily administered vaccine that can confer protection from cholera is needed for both children and adults in developing countries. One candidate, Peru-15 is a live oral vaccine that is based on an attenuated mutant of a *Vibrio cholerae* O1, El Tor Inaba strain. The vaccine has been found to be safe, immunogenic, and efficacious in North American volunteers [1–3] and safe and immunogenic in Bangladeshi adults [4]. Peru-15 genetically engineered to be non-toxinogenic and non-recombinational. It is non-motile and ctxB positive [2] and genetically stable [1,4]. Since the vaccine is immunogenic in Bangladeshi and naïve North American volunteers it may likewise be immunogenic in children who have not been primed with *V. cholerae* O1. After satisfactory immunogenicity and safety studies in adults [4], we initiated studies in descending age groups from toddlers to infants 9 months of age to evaluate safety, immunogenicity and excretion of the vaccine strain in Phase I/Phase II studies in Bangladesh.

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2. Subjects and methods

2.1. Study participants

The study participants were children from an urban slum in the Mirpur area in Dhaka city in Bangladesh. The inpatient facility was adjacent to the ICDDR,B hospital. The outpatient facility was at the Mirpur field site, in an urban neighborhood about 10 km from the ICDDR,B and participants were recruited from an area of about 1 km around this facility.

2.2. Eligibility

Inclusion criteria included being healthy, aged between 5 years and 9 months of age. Exclusion criteria included any chronic disease, or any recent illness, immunosuppressive conditions in the past 6 months that may compromise the immune system. Those with history of diarrheal illness (passage of loose or watery stool $\geq$3/24 h) in the past 2 weeks, febrile illness in the last week or antibiotic treatment within 2 weeks or who received any enteric vaccine within 1 month were excluded. Children whose stool sample was found to be positive for common enteric pathogens including enterotoxigenic *E. coli* were also not included [5]. For this purpose, stools were tested 3–5 days prior to immunization for the enteric pathogens using routine microscopic examination and microbiological culture. Of the eligible children (Tables 1 and 2), 240 were sequentially recruited into the study based in the order in which they were screened and then randomized into vaccine and placebo groups. Of the children screened 234 were not found to be eligible because of high parasitic load in stool and/other enteric pathogens in stool ($n = 183$), history of recent illness ($n = 50$) or undernutrition (≤ -2S.D.) ($n = 1$). Similar criteria were used for inpatient and outpatient subjects. Consent was obtained from guardians or parents to allow their child to participate in the study.

2.3. Study design

The trial was individually randomized, double blinded and placebo controlled. Randomization was carried out by the International Vaccine Institute and sent to the vaccine formulation team. The sample size calculation was carried out at a power of 90% and significant difference level at 95% and was based on observing a statistically significant difference if 70% of vaccine recipients and 10% of placebo recipients developed a four-fold rise in vibriocidal antibodies after receiving the study agent [1]. The study was monitored by an independent Data Safety Monitoring Board (DSMB).

The study was carried out using six separate groups of participants studied sequentially from the toddlers (2–5 years old) to the infants (for the sake of simplicity those between 23 and 9 months old) from the reduced to the full dose of Peru-15 and from the inpatient to the outpatient phases (Table 1). The DSMB approved the progression of the study from one phase to the next.

2.4. Study agents, allocation and administration

The freeze dried vaccine was supplied by AVANT Immunotherapeutics Inc. (Needham, MA, US). Vaccine dose was either a 10 times reduced dose of 2×10^7 cfu or a full dose of 2×10^8 cfu. The vaccine was formulated in 5 ml of chlorine free bottled water and then mixed with 45 ml of buffer (for toddlers) or 20 ml (for infants) of a bicarbonate and ascorbic acid buffer (2.5 g of bicarbonate and 1.65 g of ascorbic acid were reconstituted in 100 ml of water; this preparation was used for formulation of the vaccine for toddlers and infants). The placebo consisted of 50 or 25 ml of buffer only in the two age groups. The vaccine formulation team prepared and blinded the vaccine and the placebo according to the randomization list. The study agents were administered within an hour of preparation and the participants were not allowed to eat or drink for 60 min before and after the intake of the study agents.

2.5. Follow-up for adverse events

Participants stayed at the inpatient facility for 12 days, starting 1 day prior to vaccination. Clinical monitoring was carried out 1 h before and after intake of the study agent(s)

Table 1
Study subjects in the different phases of the Phase I/Phase II trial on Peru-15

Participants and study agent	Inpatient group (Phase I)[a] ($n = 120$)		Outpatient (Phase II)[d] ($n = 120$)
	Reduced dose[b] ($n = 60$) (2×10^7 cfu/dose)	Full dose[c] ($n = 60$) (2×10^8 cfu/dose)	Full dose ($n = 120$) (2×10^8 cfu/dose)
Toddlers			
Vaccine group	20	20	30
Placebo group	10	10	30
Infants			
Vaccine group	20	20	30
Placebo group	10	10	30

The Peru-15 Phase I/Phase II trial was carried out using six separate groups of participants studied sequentially, first in the toddlers (2–5 years) and then in the infants (9–23 months). The study was conducted in the [a]inpatient toddler group given the [b]reduced dose, followed by the [c]inpatient toddler group given the full dose and then the [d]outpatient older group given the full dose. Following the completion of the study a similar sequence was followed in the infants. The DSMB approved each phase of the study, following which the next phase was initiated.