Vaccine 34 (2016) 1551–1558

Contents lists available at ScienceDirect

Vaccine

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

The oral cholera vaccine Shanchol™ when stored at elevated temperatures maintains the safety and immunogenicity profile in Bangladeshi participants

Amit Saha[a,b], Arifuzzaman Khan[a], Umme Salma[a], Nusrat Jahan[a], Taufiqur Rahman Bhuiyan[a], Fahima Chowdhury[a], Ashraful Islam Khan[a], Farhana Khanam[a], Sundaram Muruganandham[e], Sreeramulu Reddy Kandukuri[e], Mandeep Singh Dhingra[e], John D. Clemens[a,d], Alejandro Cravioto[c], Firdausi Qadri[a,*]

[a] International Centre for Diarrhoeal Disease Research Bangladesh (icddr,b), Dhaka, Bangladesh
[b] University of New South Wales, SPHCM, NSW, Australia
[c] Global Evaluative Sciences USA, Inc., Seattle, WA 98109, United States
[d] UCLA Fielding School of Public Health, Los Angeles, United States
[e] Shantha Biotechnics Private Limited, Hyderabad, India

ARTICLE INFO

Article history:
Received 20 December 2015
Received in revised form 3 February 2016
Accepted 4 February 2016
Available online 16 February 2016

Keywords:
Shanchol™
Oral cholera vaccine
Immune response
Thermal stability
Cold chain

ABSTRACT

Background: The oral cholera vaccine (OCV), Shanchol™ has shown protective efficacy lasting up to 5 years, however, requirement for a cold chain limits its use in resource poor settings. The study was conducted to determine the safety and immunogenicity of Shanchol in adult participants in Bangladesh when stored at elevated temperatures.

Methods: The study was conducted in Mirpur, Dhaka. Four groups of healthy adult participants received two doses of Shanchol™, kept under standard storage temperature (Group A; 2–8 °C) or at elevated temperatures (Group B, 25 °C; Group C, 37 °C; Group D, 42 °C) for 14 days, respectively. Vaccine specific antibody responses were determined.

Findings: 145 participants were assigned to each group. Adverse events were mild not differing among groups. Vaccine stored at elevated temperatures remained stable with cumulative LPS content within admissible limits.

Vibriocidal antibody responses were observed in all groups after each dose of vaccine at day 7 and 21 compared to pre-immune levels ($P < 0.001$). Four-fold increases to *Vibrio cholerae* O1 Ogawa were observed at day 7 and/or day 21 after vaccination in the standard temperature and the three elevated temperature groups, with responder rates of; 76% (95% CI LB; 70%), 80% (95% CI LB; 74%), 69% (95% CI LB; 63%), and 74% (95% CI LB; 68%) in Groups A–D, respectively ($P = 0.240$). Responses were also seen in all groups to *V. cholerae* O1 Inaba and *V. cholerae* O139 and in LPS specific IgA response to *V. cholerae* O1 antigens.

Interpretation: This is the first report to show that the OCV is stable at elevated temperatures, and the safety and immunogenicity profiles are not altered. This information will help formulate global policies for use of the vaccine at higher temperatures, resulting in easier distribution and vaccination costs and decrease logistical challenges to vaccine delivery.

Funding: Bill & Melinda Gates Foundation.

Trial registration: Clinical Trials.gov number NCT01762930.

1. Introduction

Over the past 30 years cholera has been spreading globally with an increasing trend, causing epidemics. Immunization against cholera is now recognized as a major intervention for control of the disease [1,2]. Recently large studies have been carried out with an

* Corresponding author at: International Centre for Diarrhoeal Disease Research (icddr,b), 68, Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka 1212, Bangladesh. Tel.: +880 2 9827001 10x2431/2 9827035; fax: +880 2 9827062/9827070.
E-mail address: fqadri@icddrb.org (F. Qadri).

http://dx.doi.org/10.1016/j.vaccine.2016.02.020
0264-410X/

affordable oral, killed whole cell cholera vaccine (OCV), Shanchol, and have provided evidence of substantial protective efficacy [3–7] lasting for up to 5 years after immunization [3]. However, even though the vaccine has several important attributes which make it useful for delivery to age-groups, one year and above, the vaccine's label mandates for cold chain storage until delivery at 2–8 °C, which can hinder its use in certain resource poor settings where cholera is prevalent. Globally cholera is seen in warm settings where ambient temperatures can exceed 40 °C. Shanchol consists of heat and formalin inactivated whole cell components of *Vibrio cholerae* O1 and O139; the major protective antigen, LPS, is heat stable [7]. Therefore, it should be possible to use the vaccine outside the cold chain of 2–8 °C without changes to its stability, safety and immunogenicity profile.

Temperature stability studies of vaccines under the EPI (Expanded Program for Immunization) have been carried out by national immunization programs as well as for hepatitis B vaccine [8,9]. The recommended storage temperature of Shanchol, 2–8 °C has been maintained in studies in India, Bangladesh and Haiti [10–13]. It is therefore very important to determine the effect of elevated storage temperatures on the stability, safety and immunogenicity profile of Shanchol. The present paper reports on such a study conducted in adult participants in Bangladesh.

2. Methods

2.1. Field site and study population

The study was conducted in 580 healthy participants aged 18–45 years in Mirpur, Dhaka, Bangladesh [5,14]. Participants consenting to the study were enrolled based on exclusion criteria: fever or gastrointestinal disorder in past 7 days, recent receipt of anti-diarrheal medication, known chronic illness or immunocompromised, pregnancy, or earlier receipt of OCV.

2.2. Ethical considerations

The protocol was approved by the IRB of the icddr,b. Written informed consent was obtained from all participants. The study was registered as ClinicalTrials.gov number, NCT01762930.

2.3. Sample size

The study was designed as a non-inferiority trial, whose primary aim was to exclude inferior immune responses in the higher temperature storage groups relative to the standard temperature storage group. Assuming a true immunogenicity of vaccine with standard temperature of 60% and immunological response at least 40% in recipients in different groups (25 °C, 37 °C and 42 °C), to exclude a standard-experimental temperature difference of 5% (lower boundary of the 1-tailed 95% confidence interval) in immunogenicity with a power of 80%, the minimum number of participants required for each group was 132 and with 10% attrition, the number was 145 in each of the 4 groups A, B, C and D.

2.4. Study intervention: oral cholera vaccine (Shanchol)

The study was a non-blinded open study. The investigators in the laboratory carrying out immunological assays were blinded as to the allocation using the OCV, Shanchol. Each dose of the Shanchol vaccine is remain in a 1.5 mL vial, contained heat killed and formalin killed whole cell bacteria consisting of 600 ELISA units (EU) of lipopolysaccharide (LPS) of formalin-killed *V. cholerae* O1 Inaba, El Tor strain Phil 6973, 300 EU LPS of heat-killed *V. cholerae* O1 Ogawa classical strain Cairo 50; 300 EU LPS of formalin killed *V. cholerae* O1 Ogawa classical strain Cairo 50; 300 EU LPS of heat-killed *V. cholerae* O1 Inaba, classical strain Cairo 48; and 600 EU LPS of formalin killed *V. cholerae* O139 strain 4260B [13]. The Shanchol is suspended in a buffer known as the Oral Cholera Vaccine Diluent (OCVD) which is composed of sodium phosphate.

2.5. Vaccine allocation

The vaccine vials were labeled based on the temperature at which they had been incubated and grouped as A–D (Group-A, 2–8 °C; Group-B, 25 °C; Group-C, 37 °C and Group-D, 42 °C).

2.6. Study procedures

Shanchol™ was incubated at 25 °C, 37 °C and 42 °C for 14 days in incubators, calibrated to the specified temperatures. Following this, vaccines were stored at 2–8 °C until used for the immunizations. To measure the LPS content after keeping at different temperatures, a set of 5 vaccine vials from each group was analyzed at Shantha Biotechnics Private Limited, Hyderabad, India. Two doses of vaccine were administered at a 14-day interval [13].

The study participants were monitored 14 days after each vaccination to assess the safety of the study agent. After each dose of vaccine, the recipients were observed for 30 min in the field clinic by a study physician. Trained study staff visited the participants' homes daily for the first 3 days post vaccination to record adverse events such as diarrhea, vomiting, nausea and other local and systemic reactions. For the remaining 11 days, all participants were instructed to visit the field clinic to record if they had any adverse events. The same schedule was maintained after the second dose. Diarrhea was defined as 3 or more loose or watery stools within a 24 h period. An adverse event is defined as an untoward medical event with an onset up to 14 days after receipt of a dose which may or may not be associated with the vaccine and serious adverse events as any untoward medical occurrence as has been carried out earlier [15].

Blood samples were obtained at day 0 prior to the first dose of vaccine and 7 days after each dose of vaccine at day 7 and at day 21. Acute responses to the vaccine were measured by serum vibriocidal antibodies and *V. cholerae* O1 Ogawa and Inaba LPS specific antibodies using serum specimens collected at the 3 time points [16–19].

2.7. Method of vaccine LPS content measurement

Shanchol vaccine is consist of inactivated strains of O1 Inaba Cairo 48, O1 Ogawa Cairo 50, O139 4260B and O1 Inaba El Tor Phil 6973. The LPS content of each strain in monovalent bulks and finished product (vaccine) is quantitatively measured by an inhibition ELISA. Strain specific purified LPS antigen is used as a coating reagent and the coated plates incubated for ~20 h. Three fold dilutions of test samples and LPS standard are prepared in another 96 well plate. To these standard and sample preparations, strain specific primary antibodies are added and incubated to form Antigen–Antibody complexes. These Antigen–Antibody complexes suspension is transferred to the first coated plate to facilitate for binding of leftover primary antibodies to the LPS antigen. Strain specific primary antibodies bind to the specific LPS antigen to form complex. To this Ag–Ab complex, Horseradish peroxidase (HRP) conjugate binds and this reaction forms a color by the addition of tetramethylbenzidine (TMB) substrate. Higher the concentration of the primary antibodies the stronger the color change. Color strength is quantitatively measured by a spectrometer. Absorbance values of these preparations are used for the calculation of LPS content in L.E.U./mL (LPS ELISA units per mL).