Vaccine 32 (2014) 7077–7084

Contents lists available at ScienceDirect

Vaccine

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

Safety and immunogenicity of an improved oral inactivated multivalent enterotoxigenic *Escherichia coli* (ETEC) vaccine administered alone and together with dmLT adjuvant in a double-blind, randomized, placebo-controlled Phase I study☆

Anna Lundgren[a,*], Louis Bourgeois[b], Nils Carlin[c], John Clements[d], Björn Gustafsson[c], Marianne Hartford[e], Jan Holmgren[a], Max Petzold[f], Richard Walker[b], Ann-Mari Svennerholm[a]

[a] University of Gothenburg Vaccine Research Institute (GUVAX), Dept. of Microbiology and Immunology, University of Gothenburg, Box 435, 405 30 Gothenburg, Sweden
[b] PATH, 455 Massachusetts Ave, NW, WA, DC 20001, USA
[c] Scandinavian Biopharma, Gunnar Asplunds allé 16, 171 63 Solna, Sweden
[d] Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA 70112, USA
[e] Clinical Trial Center, Sahlgrenska University Hospital, Gröna stråket 12, 413 45 Gothenburg, Sweden
[f] Centre for Applied Biostatistics, Sahlgrenska Academy, University of Gothenburg, Box 414, 405 30 Gothenburg, Sweden

ARTICLE INFO

Article history:
Received 8 August 2014
Received in revised form 24 October 2014
Accepted 27 October 2014
Available online 5 November 2014

Keywords:
ETEC
Vaccine
dmLT
IgA
Intestinal immunity
Human

ABSTRACT

Background: We have developed a new oral vaccine against enterotoxigenic *Escherichia coli* (ETEC), which is the most common cause of bacterial diarrhea in children in developing countries and in travelers.
Methods: The vaccine was tested for safety and immunogenicity alone and together with double-mutant heat-labile toxin (dmLT) adjuvant in a double-blind, placebo-controlled Phase I study in 129 Swedish adults. The vaccine consists of four inactivated recombinant *E. coli* strains overexpressing the major ETEC colonization factors (CFs) CFA/I, CS3, CS5, and CS6 mixed with an LT B-subunit related toxoid, LCTBA. Volunteers received two oral doses of vaccine alone, vaccine plus 10 μg or 25 μg dmLT or placebo. Secretory IgA antibody responses in fecal samples and IgA responses in secretions from circulating intestine-derived antibody secreting cells were assessed as primary measures of vaccine immunogenicity.
Results: The vaccine was safe and well tolerated; adverse events were few and generally mild with no significant differences between subjects receiving placebo or vaccine with or without adjuvant. As many as 74% of subjects receiving vaccine alone and 83% receiving vaccine plus 10 μg dmLT showed significant mucosal IgA responses to all five primary vaccine antigens and about 90% of all vaccinees responded to at least four of the antigens. Subjects receiving vaccine plus 10 μg dmLT responded with significantly increased intestine-derived anti-CS6 responses compared to subjects receiving vaccine alone.
Conclusions: The vaccine was safe and broadly immunogenic. dmLT further enhanced mucosal immune responses to CF antigens present in low amounts in the vaccine. Based on these encouraging results, the vaccine will be tested for safety and immunogenicity in different age groups including infants in Bangladesh and for protective efficacy in travelers.

Abbreviations: AE, adverse event; ALS, antibodies in lymphocyte supernatants assay; ASC, antibody secreting cell; CF, colonization factor; CT, cholera toxin; CTB, cholera toxin binding subunit; dmLT, double mutant LT; ETEC, enterotoxigenic *Escherichia coli*; GM, geometric mean; LT, heat labile toxin; LTB, heat labile toxin binding subunit; LCTBA, CTB/LTB hybrid protein; MEV, multivalent ETEC vaccine; mLT, single-mutant LT; PBMCs, peripheral blood mononuclear cells; PPS, per protocol analysis set; SAS, safety analysis set; ST, heat-stable toxin; SIgA, secretory IgA.

☆ Clinical trials registration: ISRCTN91363076.

* Corresponding author. Tel.: +46 31 7866213; fax: +46 31 7866205.
E-mail addresses: anna.lundgren@microbio.gu.se (A. Lundgren), lbourgeois@path.org (L. Bourgeois), nils.carlin@etvax.se (N. Carlin), jclemen@tulane.edu (J. Clements), bjorn.gustafsson@etvax.se (B. Gustafsson), amhgbg@gmail.com (M. Hartford), jan.holmgren@microbio.gu.se (J. Holmgren), max.petzold@gu.se (M. Petzold), rwalker@path.org (R. Walker), ann-mari.svennerholm@microbio.gu.se (A.-M. Svennerholm).

http://dx.doi.org/10.1016/j.vaccine.2014.10.069

1. Introduction

Although enterotoxigenic *Escherichia coli* (ETEC) is the most frequent bacterial cause of diarrhea in children in developing countries and the major cause of travelers' diarrhea, no vaccine is yet available against ETEC disease [1–3]. ETEC disease occurs after ingestion of ETEC leading to bacterial colonization of the intestinal mucosa by means of surface-expressed colonization factors (CFs) on the bacteria and production of a heat-labile toxin (LT) and/or a heat-stable toxin (ST) that induce watery diarrhea [3,4]. Immune protection is mediated by anti-CF and/or anti-LT antibodies produced locally in the intestine [2,5].

We have previously developed an oral vaccine consisting of inactivated ETEC bacteria expressing prevalent CFs and recombinantly produced cholera toxin binding subunit (CTB) [5,6]. This vaccine was shown to be safe and immunogenic in children and adults in endemic areas and conferred protection against moderate/severe diarrhea in adult travelers [5,7]. However, the protective efficacy in developing-country children was not significant and a full dose of vaccine, but not a quarter dose, induced vomiting in children 6–17 months old [2,8]. Therefore, we have now developed a modified second-generation oral ETEC vaccine with the aim to improve its immunogenicity without increasing the dosage and to be able to give a reduced dose to infants [5,9]. Our approach has been to construct recombinant *E. coli* strains expressing increased amounts of the most prevalent CFs [10] and to include a CTB/LTB hybrid protein (LCTB*A*), which induces stronger anti-LT responses than CTB in both mice and humans [11,12]. We have also broadened the coverage of the vaccine by including a strain expressing the prevalent colonization factor CS6 in immunogenic form [13]. This new multivalent ETEC vaccine (MEV) contains four different inactivated *E. coli* strains expressing substantially higher levels of CFA/I, CS3, CS5 and CS6 than in the first-generation vaccine, plus LCTB*A* [9]. In addition, we have evaluated the possibility to further enhance the immunogenicity of the vaccine by coadministration with the double-mutant LT (dmLT) adjuvant [14]. Our preclinical studies have demonstrated that addition of dmLT to MEV significantly improved both the anti-CF and anti-LT responses following oral immunization [9].

The primary objectives of this study were to evaluate the safety and mucosal immunogenicity of MEV and to explore if the immunogenicity of the vaccine might be further enhanced by addition of dmLT adjuvant. Serum anti-LT and toxin-neutralizing immune responses were determined as secondary and exploratory measures. These aspects were addressed in a Phase I clinical trial including 129 adult Swedish volunteers given either vaccine alone or together with two different dosages (10 μg and 25 μg) of dmLT; a matched control group received buffer only.

The results show that the vaccine was safe and well tolerated, both when given alone and in combination with dmLT adjuvant. The vaccine induced significant mucosal and intestine-derived immune responses to all major vaccine antigens and dmLT further enhanced mucosal immune responses to the CF antigens present in low amounts in the vaccine.

2. Materials and methods

For additional information, see Supplementary material.

2.1. Study design

This was a four-armed, randomized, double-blind, placebo-controlled, single-center Phase I trial. The study was approved by the Ethical Review Board in the Gothenburg Region, the Western Institutional Review Board, USA and the Swedish Medical Product Agency.

2.2. Randomization and masking

Healthy adult subjects, 18 to 43 years, were randomized into one of four groups (A–D); each group was given two oral doses two weeks apart of one of the following treatments: (A) vaccine buffer alone ($n = 34$), (B) MEV alone ($n = 35$), (C) MEV plus 10 μg dmLT ($n = 30$) or (D) MEV plus 25 μg dmLT ($n = 30$). A computer-generated randomization list was prepared by a statistician otherwise not involved in the study.

2.3. Vaccine

MEV (also called Etvax) consists of four inactivated recombinant *E. coli* strains (ETEX 21–24) which overexpress CFA/I, CS3, CS5 and CS6, respectively, mixed with LCTB*A* [9]. The CFA/I, CS3 and CS5 expressing strains, all based on a toxin-negative O78 ETEC strain, were inactivated with formalin and the CS6 expressing *E. coli* K12 strain with phenol to retain CF expression on the bacterial surface [10,13]. LCTB*A* is a recombinantly produced LTB/CTB hybrid protein in which seven amino acids in CTB have been replaced by corresponding amino acids of LTB [12]. dmLT (R192G/L211A) is an LT-derived protein which contains two genetic substitutions in the A subunit which eliminates the enterotoxic activity without removing the adjuvant activity [14].

2.4. Procedures

Volunteers received two oral doses of vaccine ± dmLT in bicarbonate buffer or placebo (buffer alone) two weeks apart (day 0 and day 14 ± 2). Fecal samples were collected on days 0, 7 ± 1, 14 ± 2, 19 ± 1, 21 ± 1 and 28 ± 2, blood samples for isolation of peripheral blood mononuclear cells (PBMCs) on days 0, 7 ± 1, 19 and 21 ± 1 and serum samples on days 0, 7 ± 1, 14 ± 2, 19 ± 1, 21 ± 1, 28 ± 2 and 40–56.

Safety was determined by evaluation of adverse event (AE) reports (diary cards and interviews) from day 0 until day 40–56, by clinical chemistry and hematology tests performed at screening and on days 7 ± 1 and 21 ± 1 and by physical examination at screening and on day 40–56. Solicited AEs listed in the study diaries were gastrointestinal symptoms (i.e. abdominal pain, nausea, vomiting, diarrhea, loose stools) plus fever.

Mucosal immune responses were evaluated by measuring intestine-derived antibody secreting cells (ASCs) and intestinal secretory IgA (SIgA) responses in fecal extracts. Systemic immune responses were analyzed by measuring serum antibody levels. PBMCs were isolated and used for ASC analyses by the antibodies in lymphocyte supernatants (ALS) and ELISPOT assays as described [11]. ASCs were detected by the ELISPOT technique using plates coated with in-house purified CFA/I, CS3, CS5 or GM1 ganglioside plus LTB or CS6 (Gift from F. Cassel) [6,11]. Fecal samples were immediately frozen at home by the subjects; fecal extracts were subsequently prepared and stored at −70 °C [11]. Antibody levels in ALS specimens, fecal extracts and sera were analyzed by ELISA using plates coated with CFA/I, CS3, CS5, CS6, GM1 plus LTB or O78 LPS [9,11]. Fecal antibody levels were determined as the antigen-specific SIgA titer divided by the total SIgA concentration of each sample [15]. LT toxin neutralization titers were determined using the Y1 adrenal cell assay [16].

2.5. Endpoints and statistical analyses

Safety endpoints were defined as absence of any vaccine-related serious AEs and not significantly higher frequencies of