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ScienceDirect
Vaccine 25 (2007) 4392–4400
Vaccine
www.elsevier.com/locate/vaccine

Randomised, double-blind, safety and efficacy of a killed oral vaccine for enterotoxigenic *E. Coli* diarrhoea of travellers to Guatemala and Mexico

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Received 27 October 2006; received in revised form 16 March 2007; accepted 20 March 2007
Available online 4 April 2007

Abstract

We tested the efficacy of a killed oral vaccine for enterotoxigenic *Escherichia coli* (ETEC) diarrhoea to determine if two doses of vaccine with colonization factor antigens (CF) and cholera B subunit would protect against ETEC diarrhoea of travellers. Six hundred seventy-two healthy travellers going to Mexico or Guatemala were studied in a prospective, randomised, placebo-controlled trial. The primary outcome was a vaccine preventable outcome (VPO), defined as an episode of ETEC diarrhoea with an ETEC organism producing heat labile toxin (LT) or CF homologous with the vaccine, without other known causes. The vaccine was safe and stimulated anti-heat labile toxin antibodies. There was a significant decrease in more severe VPO episodes (PE = 77%, $p = 0.039$) as defined by symptoms that interfered with daily activities or more than five loose stools in a day, although the total number of VPO events did not differ significantly in the vaccine and placebo groups. We conclude that the new oral ETEC vaccine reduces the rate of more severe episodes of traveller's diarrhoea (TD) due to VPO-ETEC, but it did not reduce the overall rate of ETEC diarrhoea or of travellers' diarrhoea due to other causes.

Keywords: Enterotoxigenic *E. coli*; Travellers' diarrhoea; Vaccine; Guatemala; Mexico; Oral vaccine; Colonization factor antigens; Travel

1. Introduction

Travellers' diarrhoea (TD) is a self-limited illness that usually resolves spontaneously within a few days, but it has the potential for interfering with a meticulously planned business or pleasure trip. Annually about 80 million people travel from industrialized countries to a developing country. Among them, TD had an incidence rate of 25–60% per 2 weeks stay [1,2]. TD is characterised by watery diarrhoea and may be associated with abdominal pain, cramps, nausea, vomiting, muscle aches, weakness and sometimes low-grade fever. Occasionally higher fevers (>38 °C [101 °F]) and blood in the stool occurs, but when these symptoms occur, the illness is said to be dysentery and not "ordinary" traveller's diarrhoea. TD may be caused by a variety of infectious agents, but the most common cause is an intestinal infection with

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doi:10.1016/j.vaccine.2007.03.034

Escherichia coli that are able to produce enterotoxins. These are termed enterotoxigenic *E. coli* (ETEC) [3]. Two different toxins have been described; a heat labile toxin (LT) and a heat stable toxin (ST). Strains of ETEC may produce either one or both of these toxins. In addition, most ETEC have colonization factor antigens (CFs) that facilitate their intestinal colonization [3–5]. It is thought that most pathogenic ETEC strains must be able to colonise the intestine in order to grow to high concentration in the small intestine; only then can the large numbers of bacteria produce sufficient amounts of LT and/or ST to induce illness.

Travellers become infected with these ETEC through ingestion of contaminated food and water. In theory, persons who are careful with their intake should be able to avoid the illness; however, in practice, TD remains common even in persons who are careful. Antibiotics, such as doxycycline, norfloxacin or ciprofloxacin [6–9] can reduce the rate of illness but most experts feel that prophylactic antibiotics should be avoided whenever possible.

An oral vaccine has been developed for ETEC that has been found to be safe and immunogenic in volunteers in Sweden, Egypt and Bangladesh, and was found to be efficacious in preliminary studies of travellers [10–13]. The vaccine is patterned after a similar efficacious killed oral vaccine for cholera [14] and consists of formalin-killed ETEC bacteria plus a recombinant form of the B subunit of cholera toxin. The bacteria included in the vaccine were chosen to represent the most prevalent known CFs. The vaccine induces an antibody response to the CFs, and it is hoped that anti-CF immunity will block the colonization of ETEC in the gut and thus will prevent illness. The inclusion of B subunit has been shown to induce antibodies to LT, but since it lacks the active A subunit, it is completely harmless when given orally. In a large field trial in Bangladesh, immunization with a killed whole cell cholera vaccine containing B subunit provided 67% protection against diarrhoea associated with *E. coli* producing LT for at least 3 months [15]. There is evidence that anti-CF antibodies and anti-toxin antibodies protect in a synergistic manner and the combination of the anti-CF and anti-toxin immunity may induce greater protective immunity than either alone [16]. There is currently no safe, yet immunogenic ST toxoid able to induce antibodies to the ST toxin, and the candidate vaccine used in this study is not expected to have anti-ST properties.

A successful vaccine could potentially benefit two groups, including those susceptible to TD, but it could be of even greater public health benefit to infants living in developing countries who also experience high rates of ETEC diarrhoea. This use has been studied in Egypt and Bangladesh [17,18]. This study was conducted to evaluate the efficacy of the vaccine against ETEC diarrhea caused by strains with antigens homologous with the vaccine in travellers going to geographic areas where ETEC is known to occur commonly.

2. Methods and materials

2.1. Overview of study

This was a prospective, double-blind, randomised, placebo-controlled trial of the killed oral ETEC vaccine in persons travelling from the United States to Antigua, Guatemala or Cuernavaca, Mexico. The participants in the study were recruited in the United States and received two doses of the vaccine before departure and were followed for up to 28 days in Guatemala or Mexico for the occurrence of diarrheal illnesses. The primary objective of the trial was to determine the efficacy of the vaccine in reducing episodes of diarrhoea due to ETEC that could plausibly be prevented by the vaccine (vaccine preventable outcome or VPO). A secondary objective was to evaluate the safety of the vaccine in this group of travellers. Fig. 1 shows the overall plan for vaccination and follow-up of the participants.

2.2. Eligibility criteria

Participants in the study were healthy males or females, ≥17 years of age at the time of the first vaccination, who were planning to travel to one of the surveillance sites (Antigua, Guatemala or Cuernavaca, Mexico) with plans to stay at the study site for at least 14 days, generally for language study. They had to have a local medical provider who signed a physical exam form indicating good general health, live in a household with a telephone in the US, be willing and able to comply with the protocol, and have signed the consent form after passing a test to document their understanding of the protocol. Females were not pregnant as documented by a self-administered urine pregnancy test immediately prior to the first dose of vaccine, and were willing to use a reliable birth control method during the study. The negative pregnancy test strip was sent to the Johns Hopkins University Vaccine Testing Unit (VTU) for confirmation. Persons were excluded from the study if they had clinically significant acute or chronic gastrointestinal disease, any serious medical condition, a medical condition associated with immunodeficiency, planned use of antibiotics during the trip. The protocol did not prohibit the use of antibiotics as treatment for severe diarrhoea, but specimens were obtained before starting them. To exclude persons with recent exposure to ETEC, they were also excluded if they had travelled to a developing country within the previous 1-year.

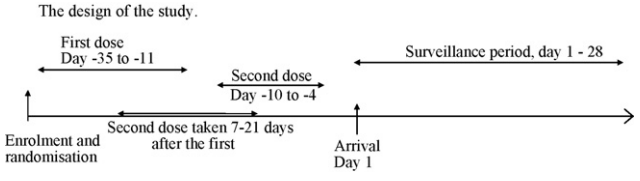

Fig. 1. The design of the study.