Clinical Evaluation of Travelan® an Oral Prophylactic for Prevention of Travelers' Diarrhea in Active Duty Military Service Assigned Abroad.

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Introduction

Infectious diarrhea is one of the most common illnesses reported by travelers and among deployed US troops visiting developing countries. Travelers' diarrhea (TD) is often acquired through ingestion of contaminated food and water. TD is predominantly bacterial, with diarrheagenic *Escherichia coli*, including enterotoxigenic (ETEC) and enteroaggregative *E. coli* (EAEC), *Campylobacter, Shigella*, and *Salmonella* species most common etiology. The morbidity associated with diarrheal illness can result in lost duty days, decreased performance, effects on judgment, decreased morale and reduced operational readiness.

ETEC is the leading cause of TD and represents an undeniable burden for US troops while deployed. TD, is currently managed symptomatically, with antibiotics recommended for more severe disease; however, given the emerging incidence of antibiotic resistance, primary prevention approaches are urgently required. Despite efforts to reduce disease by controlling food and water sources this has only been partially effective, research is focused on developing a vaccine for TD, there are however currently no licensed vaccines available in the United States. New strategies are needed to circumvent ETEC-attributable TD and a broadly active, safe, and effective oral immunoprophylaxis represents an appealing approach for travelers' and the military.

Colostrum, the first milk expressed after birthing is rich in antibodies (immunoglobulins) and innate immune components for protection of newborns against infectious agents. Travelan is a hyperimmune bovine colostrum produced by immunization of cows during gestation with a vaccine consisting of antigens derived from multiple ETEC strains known to cause TD. Travelan is a pasteurized, lactose-reduced, low-fat, high-protein powder which contains over 80% proteins by weight of which approximately 35% to 45% are antibodies. The manufacturing process involves spray drying of the colostrum to form a powder and tableting in accordance with Good Manufacturing Practices.

NMRC and USAMD-AFRIMS are working with Immuron Ltd on a Research and Clinical Development Program, the focus is on understanding, developing, and informing strategies for the protection of Defense Force personnel against infectious diarrhea. While previous clinical efficacy data with Travelan have demonstrated protection in Controlled human infection model studies (CHIM), such a regimen is cumbersome for military deployed in austere environments, as miltary field studies have shown that compliance is low with products dosed more than once per day. We present our recent in vitro and in vivo data characterizing the antibodies in Travelan and our plans to investigate a dosing regime more suitable for the military in remote locations.

Objectives

Cross-reactivity of Travelan in vitro and in vivo: Laboratory research studies have demonstrated antibodies in Travelan are reactive against ETEC strains other than those present in the bovine vaccine. Here we describe studies designed to further characterize Travelan cross reactivity and to measure the pre-clinical efficacy of Travelan in a *Shigella* challenge model in vivo.

***Clinical study to assess the protective efficacy of Travelan*:** Travelan has demonstrated clinical efficacy in preventing ETEC-attributable diarrhea in two controlled human infection model (CHIM) studies. These studies showed dosing at 200 mg or 400 mg three times a day, resulted in 84%- to over 90% protection (Otto et al., 2011). A CHIM study with a dosing schedule better suited to the military is planned to assess the efficacy of Travelan against moderate-to- severe diarrhea following challenge with ETEC strain H10407.

Materials and Methods

Immunoreactivity of Travelan antibodies with isolated TD pathogens: Whole cell lysates of 60 ETEC, 60 *Shigella* and 60 *Campylobacter* clinical isolates from several countries (Bhutan, Cambodia, Nepal and Thailand) were analyzed by western blotting by probing with Travelan. A skim milk solution was used as a control to probe replicate membranes. A similar study was performed with whole cell lysates of 71 clinical isolates of *Vibrio cholera* from Bangladesh, Cambodia and Thailand.

Efficacy of Travelan in a Shigella challenge model: Travelan (500 mg) was delivered intragastrically twice daily over 6 days to 8 naïve juvenile rhesus macaques (NJRM; *Macca mulatta*). Control animals (n=4) received a skim milk solution as placebo using the same dosing schedule. All animals were challenged with *Shigella flexneri* 2a and monitored for symptoms for up to 13 days post-challenge. Blood and fecal samples were collected daily, bacterial shedding was monitored and histological analysis of the gastrointestinal (GI) tract was performed on euthanized animals.

A randomized double-blind placebo-controlled Phase 2 clinical study:

Full details of the study plan will be presented.

Results

Travelan cross-reactive immunoreactivity with gram negative clinical isolates: Travelan demonstrated reactivity with all 60 ETEC isolates with variable staining intensity and staining patterns of reactive bands across isolates. Travelan was also reactive with all 60 *Shigella* isolates and demonstrated a dominant single reactive band in all *Campylobacter* isolates. All isolates of *Vibrio cholera* showed reactive bands when probed with Travelan compared with no staining reactivity for any of the clinical isolates when probed with skim milk.

Travelan prevents clinical shigellosis: All placebo-treated NJRMs experienced dysentery within 24 – 36 hours of challenge, whilst only 2 of the 8 (25%) Travelan-treated group developed dysentery. These results demonstrate a 75% efficacy in this in vivo *Shigella* challenge model ($p < 0.05$). Histopathological analysis revealed that all animals in the placebo-treated group displayed severe inflammation in different parts of the GI tract. These animals also had very high levels of inflammatory cytokines (IL-1b, IL-6 and IL-8) in fecal samples collected throughout the study. The inflammation seen in the GI tract and the increase in inflammatory cytokines in the feces closely correlated with the observed dysenteric clinical outcomes. Three Travelan-treated animals had signs of inflammation in the GI tract, and only 2 of those had high levels of inflammatory cytokines in fecal samples. All other animals in the Travelan-treated group were clinically healthy and did not excrete any inflammatory cytokines.

Clinical ETEC CHIM Travelan study: The status and results of the study at the time of this Symposium will be presented.

Conclusions

Travelan demonstrated wide immunoreactivity with over 200 clinical field isolates of gram-negative bacteria. This has significant implications for the use of Travelan in military (and other travelling populations) for the prevention of TD caused by multiple strains of ETEC, *Shigella, Campylobacter and Cholera Spp*. ETEC

The results from the *Shigella* challenge study results suggests that Travelan is functionally cross-reactive and may have some prophylactic activity against Shigellosis. Overall, these findings provide implications for protection of the war fighter in the field and the potential for long-term humanitarian protection and treatment for endemic populations in an outbreak of these diarrheal-pathogens. Furthermore if the dosing schedule of Travelan used in the clinical study provide implications for demonstrates protective efficacy in the ETEC-challenge study, this could lead to significant positive impacts for the future protection against TD for military units.