

Enteraggregative *Escherichia coli* Diarrhea in Travelers: Response to Rifaximin Therapy

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Background & Aims: We have recently shown that enteraggregative *Escherichia coli* (EAEC) strains commonly cause travelers' diarrhea. The study was designed to determine whether U.S. travelers with EAEC diarrhea responded to rifaximin therapy. **Methods:** In a multicenter placebo-controlled clinical trial of travelers' diarrhea without non-EAEC pathogens we evaluated 2 doses of rifaximin. EAEC was sought in stool samples in enrolled subjects by HEp-2 cell assay. Response to rifaximin (both groups combined) and placebo were evaluated in EAEC-positive and EAEC-negative patient groups. **Results:** Compared with placebo, rifaximin shortened the postenrollment illness in travelers with EAEC diarrhea (median, 22 vs. 72 hours; $P = 0.03$). In subjects with EAEC-negative diarrhea, the median duration of post-treatment diarrhea was shorter with rifaximin (33 hours) than with placebo (52 hours), but this difference was not significantly different ($P = 0.14$). **Conclusions:** Improvement of EAEC-mediated diarrhea with antibiotic treatment supports the pathogenicity of this organism in travelers to developing countries. The study provides information on the value of the poorly absorbed drug rifaximin in therapy of travelers' diarrhea.

Diarrhea is the most common health problem among international travelers to developing tropical and subtropical areas.^{1,2} Enteraggregative *Escherichia coli* (EAEC) are increasingly recognized as a cause of travelers' diarrhea, responsible for up to one fourth of cases.³ Currently, EAEC are identified by demonstration of an aggregative or "stacked brick" adhesion phenotype after culture on cell monolayers (HEp-2).⁴

Preliminary information is available to suggest that antibacterial therapy will shorten the illness of EAEC diarrhea.^{5,6} In one partially controlled study, ciprofloxacin shortened post-treatment diarrhea among international travelers from 56 hours in a placebo group to 35 hours.⁶ In a second small study, patients with acquired immunodeficiency syndrome-associated EAEC diarrhea

were shown to have their illness shortened by fluoroquinolone treatment.⁵

We have previously shown that antibacterial drugs significantly shortened the duration of travelers' diarrhea in which a definable bacterial pathogen was not found,⁷⁻⁹ but EAEC had not been sought in these studies. A multicenter placebo-controlled trial that we were carrying out in Mexico, Guatemala, and Kenya, in which we were looking at the efficacy of rifaximin in treating travelers' diarrhea,¹⁰ provided the opportunity to determine whether clinical responsiveness of cases with EAEC diarrhea to antibacterial therapy might explain our earlier finding that antibacterial therapy shortened pathogen-negative travelers' diarrhea.

Methods

The study involved 380 international tourists or college students who developed acute diarrhea shortly after traveling to Guadalajara, Mexico, Antigua, Guatemala, or Mombasa, Kenya.¹⁰ Subjects were randomized to receive rifaximin 200 mg 3 times daily or 400 mg 3 times daily or placebo for 3 days.

Diarrhea was defined as the passage of more than 3 unformed stools in 24 hours with a duration of illness of less than 72 hours. One or more additional signs or symptoms of enteric infection had to have been present, including abdominal pain or cramps, nausea, vomiting, excess intestinal gas, fecal urgency, or tenesmus. Patients provided a pretreatment stool sample, from which 5 colonies of *E. coli* per sample were collected and assayed for EAEC by using the HEp-2 cell assay,⁴ when the sample was otherwise pathogen-negative. Other definable bacterial and protozoal pathogens were also studied in the larger study¹⁰ but are not included in this study. The

Abbreviations used in this paper: CI, confidence interval; EAEC, enteraggregative *Escherichia coli*; TLUS, time to last unformed stool.

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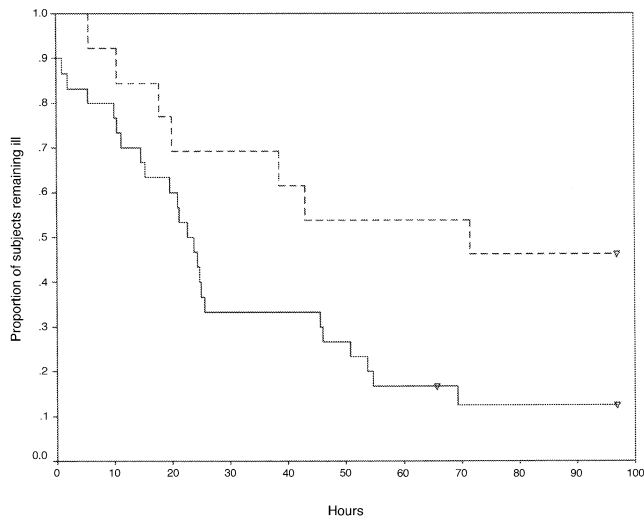


Figure 1. Kaplan–Meier estimates showing the proportion of subjects remaining ill after EAEC diarrhea who were treated with rifaximin ($n = 30$; solid line) or placebo ($n = 13$; dashed line). Censored cases represented with the symbol “▽”. Curves are significantly different in favor of rifaximin ($P = 0.03$, Wilcoxon test).

study was not designed to look for mixed infections in which the clinical response to antibacterial therapy could be difficult to interpret.

Subjects were monitored and maintained daily symptom diaries for 5 days after initiation of therapy. The primary study end point was time from initiation of medication until passage of the last unformed stool after which subjects were declared well, or time to last unformed stool (TLUS). As defined by a U.S. Food and Drug Administration subcommittee guideline,¹¹ TLUS or cure was declared when there was (1) passage of no unformed stools without fever during a 48-hour interval or (2) passage of no watery stools and no more than 2 soft stools and an absence of fever or other enteric symptoms in a 24-hour interval.¹¹

Response to treatment in cases was analyzed for 4 diarrhea treatment groups: EAEC isolated and treated with rifaximin or placebo, or no pathogen isolated and treated with rifaximin or placebo. The statistical method used to analyze the effect of therapy on TLUS was the Kaplan–Meier survival analysis of the proportion of subjects remaining ill during the study, with the Wilcoxon test for comparison of survival curves. Follow-up of subjects for 120 hours after initiation of therapy allowed the determination of TLUS within the first 96 hours. Subjects with undetermined TLUS because of early withdrawal were noted as censored at the time for which the last information was available, and those with diarrhea lasting more than 96 hours were noted as censored as of 97 hours. The data were processed by using MS Excel (Microsoft, Redmond, WA) and SAS (SAS Institute, Cary, NC). As part of the larger clinical trial, the study was approved by the University of Texas—Houston Health Science Center, Committee for the Protection of Human Subjects.

Results

One hundred thirty-seven subjects whose stool samples were negative for routinely definable pathogens were included in the study (45 from Mexico, 63 from Guatemala, and 29 from Kenya). Forty-four of the 137 (32%) subjects were identified as having EAEC in pre-treatment stools—15 (33%) from Mexico, 19 (30%) from Guatemala, and 10 (34%) from Kenya. Response to therapy did not differ by location studied, and there was not a difference in response in the 2 different rifaximin drug dosage regimens.

Figure 1 illustrates a comparison of the proportion of subjects remaining ill during the study by means of Kaplan–Meier survival analysis for rifaximin-treated ($n = 30$) and placebo-treated ($n = 13$) cases of EAEC diarrhea. Median TLUS was significantly ($P = 0.03$, Wilcoxon test) shorter for subjects with EAEC-positive travelers’ diarrhea treated with rifaximin (22 hours; 95% confidence interval [CI], 15–25 hours) compared with placebo (72 hours; 95% CI, 20–72 hours).

Figure 2 illustrates a comparison of the proportion of subjects remaining ill during the study by means of Kaplan–Meier survival analysis for rifaximin-treated ($n = 55$) and placebo-treated ($n = 39$) cases of no pathogen diarrhea. Median TLUS was shorter for subjects with pathogen-negative travelers’ diarrhea treated with rifaximin (33 hours; 95% CI, 19–47 hours) compared with placebo (52 hours; 95% CI, 40–78 hours), but this difference was not significantly different ($P = 0.14$, Wilcoxon test).

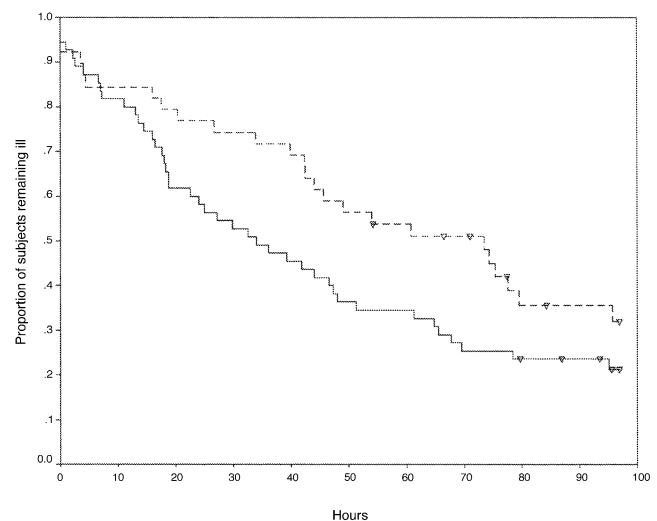


Figure 2. Kaplan–Meier estimates showing the proportion of subjects remaining ill after pathogen-negative diarrhea who were treated with rifaximin ($n = 55$; solid line) or placebo ($n = 39$; dashed line). Censored cases represented with the symbol “▽”. Curves are not significantly different ($P = 0.14$, Wilcoxon test).

Discussion

EAEC is an important emerging pathogen that has been shown to cause persistent diarrhea in young children in developing countries,^{1,2} acquired immunodeficiency syndrome-associated diarrhea,^{13,14} and travelers' diarrhea.^{3,15} It appears that EAEC rivals enterotoxigenic *E. coli* as a common cause of travelers' diarrhea and might explain up to one fourth of illness.³ EAEC is found in food in endemic areas¹⁶ and commonly produces asymptomatic infection in these areas.¹⁷ The strains recurrently infecting international travelers are highly heterogeneous and differ in virulence potential.^{17,18} The potential for contamination of tissue culture cells makes the conventional HEp-2 cell assay difficult to maintain for research laboratories. A recent advance, using formalin-treated cells to preserve them for future use,¹⁹ might be of help for laboratories wishing to detect this emerging pathogen in patients with diarrhea.

In our past studies we surmised that undetected bacterial agents would be identified later in travelers' diarrhea in view of the favorable effect of antibacterial drugs in the otherwise pathogen-negative cases.⁷⁻⁹ The present study took advantage of a multicenter study being carried out in Mexico, Guatemala, and Kenya¹⁰ to look at the possibility that EAEC infection might explain this positive response to antibacterial therapy of travelers' diarrhea without etiologic agents identified. We elected to study those without other pathogens, eliminating mixed infections in which the clinical response to antibacterial therapy would be difficult to interpret. This was a therapeutic trial rather than a study of etiology of travelers' diarrhea.

Rifaximin, an antibacterial with less than 1% absorption is known to be effective in shortening the duration of travelers' diarrhea,²⁰ shortened EAEC diarrhea from that seen in placebo-treated subjects by approximately 50 hours (from a median of 72 hours to a median of 22 hours). Clear divergence of survival curves was observed by the end of the first day after initiation of treatment. The median duration of post-treatment diarrhea (TLUS) among subjects with EAEC diarrhea treated with rifaximin (22 hours) was similar to that observed in a study comparing rifaximin and ciprofloxacin for the treatment of travelers' diarrhea (25.7 and 25.0 hours, respectively) in which enterotoxigenic *E. coli* was the predominant identified pathogen.²⁰

Rifaximin is licensed in Mexico and other countries of Europe, Asia, and Africa. The drug is used for acute bacterial diarrhea,²¹ hepatic encephalopathy,²² bacterial overgrowth syndrome,²³ and gas-related symptoms.²⁴ It is as effective as ciprofloxacin in shortening travelers'

diarrhea.²⁰ We believe that when it is available in the United States, it will be of major value in the management of bacterial diarrhea and should represent the treatment of choice for travelers' diarrhea.

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