Effect of Nitazoxanide in Diarrhea and Enteritis Caused by Cryptosporidium Species

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Background & Aims: The aim of this study was to evaluate the efficacy of nitazoxanide for the treatment of diarrhea and enteritis caused by Cryptosporidium species in patients 12 years of age and older. Methods: A multicenter, randomized, double-blind, placebo-controlled study was conducted in 90 outpatients 12 years of age and older from the Nile Delta region of Egypt. Patients were randomized to receive either one 500 mg tablet or one matching placebo tablet, or 25 mL of nitazoxanide oral suspension (500 mg nitazoxanide), each given twice daily for 3 days. Clinical and microbiologic response rates were evaluated 4 days after completion of treatment. Results: Twenty-seven (96%) of the 28 patients receiving nitazoxanide tablets responded clinically compared with 11 (41%) of 27 patients who received placebo (P < .0001). Twenty-six (93%) of the 28 patients who received nitazoxanide were free of Cryptosporidium oocysts in each of 2 posttreatment stool samples compared with only 10 (37%) of 27 patients who received placebo (P < .0001). Response rates in patients receiving the tablets and the suspension were comparable (clinical response rate for suspension, 27 of 31 [87%]; microbiologic response rate for suspension, 28 of 31 [90%]). Conclusions: These findings show that a 3-day course of nitazoxanide is effective in treating diarrhea and enteritis caused by Cryptosporidium in nonimmunodeficient patients 12 years of age and older.

The intracellular coccidian protozoan, Cryptosporidium, is a common yet underdiagnosed cause of persistent diarrhea and enteritis.1 Once considered a disease limited to persons with acquired immune deficiency syndrome, Cryptosporidium is now recognized as endemic in the United States population with 20%–65% of the population carrying antibodies, suggesting recent exposure.2,3

Similar to other enteric protozoan infections, Cryptosporidium is contracted by a fecal-oral route, usually by person-to-person contact or by ingesting contaminated food or water. The infectious dose is very low, and the oocysts of Cryptosporidium are highly resistant to chlorine. Food and person-to-person transmission may be at least as important as drinking water and may be more likely to transmit higher dose exposures. Symptoms of Cryptosporidium infection typically include diarrhea, fever, abdominal cramps, vomiting, nausea, and weight loss. The disease is typically much more severe in immunocompromised and immunodeficient patients. Factors believed to contribute to the severity of disease in a given patient include virulence of the strain, the number of organisms ingested, and the level of protective immunity. It even has been suggested that the purification of water supplies may increase the risk for cryptosporidiosis by reducing protective immunity gained from frequent but low-level exposure to Cryptosporidium.1,3–5

Cryptosporidium infection is underdiagnosed in part because of difficulties in identifying oocysts in a stool sample, but also because there has been no effective treatment, and therefore very little practical reason to diagnose the infection. Traditional antiprotozoal drugs (eg, metronidazole) have not proven to be effective in treating cryptosporidiosis.

Nitazoxanide (Alinia, Romark Pharmaceuticals, Tampa, FL) is a thiazolide anti-infective with broad-spectrum activity against enteric pathogens including protozoa, anaerobic bacteria, and viruses. It is marketed in the United States for treating diarrhea caused by Giardia lamblia and Cryptosporidium species, and it is being investigated for treatment of rotavirus diarrhea, Clostridium difficile–associated diarrhea, and Crohn’s disease.

Double-blind, placebo-controlled, clinical studies have shown that a 3-day course of nitazoxanide is effective in treating diarrhea and enteritis caused by a broad range of protozoan infections including G lamblia, Entamoeba histolytica, and Blastocystis hominis and for treating...
diarrhea caused by Cryptosporidium in nonimmunodeficient children.6–9

We report a double-blind placebo-controlled study confirming the effectiveness of nitazoxanide tablets and oral suspension in treating diarrhea caused by Cryptosporidium species in adults. Unlike an earlier study of nitazoxanide in treating diarrhea caused by Cryptosporidium in adults,9 this study was limited to patients with Cryptosporidium as the sole cause of diarrhea. The results of this double-blind placebo-controlled trial provide for a better understanding of the course of the disease in nonimmunodeficient adults and the benefits that may be obtained from diagnosis and treatment.

**Patients and Methods**

**Study Design**

We performed a prospective, randomized, double-blind, placebo-controlled study to evaluate the effectiveness of nitazoxanide tablets compared with a placebo tablet and nitazoxanide oral suspension in treating diarrhea caused by Cryptosporidium species in nonimmunodeficient patients aged 12 years and older. In the absence of guidelines for evaluation of a new drug for treating diarrhea caused by Cryptosporidium species, published guidelines for evaluation of new anti-infective drugs for treating diarrhea caused by G lamblia were consulted in designing the study.10 Patients were randomized to receive either 1 nitazoxanide 500 mg tablet or 25 mL of a 100 mg/5 mL nitazoxanide oral suspension (500 mg nitazoxanide) or 1 placebo tablet, each given twice daily for 3 days. The primary end point of the study was clinical response recorded at the day 7 follow-up visit. Clinical response was defined as well (no symptoms, no watery stools and no more than 2 soft stools, and no hematochezia within the past 24 hours or no symptoms and no unformed stools within the past 48 hours) or continuing illness. Microbiologic response, defined as either eradicated (no Cryptosporidium oocysts observed in either of 2 stool samples collected between study days 7 and 10) or persistence, was evaluated as a secondary end point. The study was designed to enroll 90 patients (30 patients per treatment group). Previous studies of nitazoxanide in treating diarrhea caused by other enteric protozoan pathogens suggested that response rates to nitazoxanide therapy using this study design should be at least 80% whereas placebo response rates should be no more than 40%. By using these assumed response rates, a sample size of 30 patients per treatment group was deemed sufficiently powerful (85%) to show that treatment with the nitazoxanide tablets or the nitazoxanide suspension is superior to treatment with placebo using a 2-sided Fisher exact test and a 5% significance level. The study was conducted in compliance with the human experimentation guidelines of the US Department of Health and Human Services. The protocol and informed consent forms were approved by the ethical committees of the participating institutions.

**Patients**

Patients presenting with diarrhea at the outpatient clinics of the Department of Hepatology, Gastroenterology and Infectious Diseases of the Benha University Hospital and the Alexandria University Hospital in the Nile Delta region of Egypt were screened for enrollment in the study. The screening was part of a broader program to identify patients for placebo-controlled studies of nitazoxanide in treating diarrhea and enteritis associated with enteric protozoa including G lamblia, E histolytica, and B hominis. Before screening, written informed consent was obtained from each of the patients, and for patients less than 18 years of age informed consent was obtained from their parents or guardians. Patients with diarrhea (≥3 bowel movements/day) and Cryptosporidium oocysts in a stool sample at screening were eligible for enrollment. Patients with other identified enteric pathogens, pregnant and lactating females, patients using any drug with antiprotozoal activity within 2 weeks of enrollment, and patients known to have or suspected of having acquired immune deficiency syndrome or other immune deficiencies were excluded from the studies.

**Assessment of Cause of Diarrhea and Enteritis**

All stool samples were subjected to a direct examination, an examination after concentration, a Ziehl–Neelsen stain, and immunofluorescence assay (MeriFluor; Meridian Diagnostics, Cincinnati, OH) for parasitic causes of diarrhea and enteritis. A stool culture was performed on the baseline stool sample to identify bacterial causes of diarrhea including adherent or toxigenic Escherichia coli.

**Study Procedures and Follow-Up Evaluation**

Patients enrolled in the study underwent a complete physical examination including recording of systolic and diastolic blood pressure, pulse rate, body weight, and temperature, and an assessment of stool characteristics (frequency, consistency, presence of mucus or blood). Patients received either 1 nitazoxanide 500 mg tablet or a matching placebo tablet or 25 mL of nitazoxanide oral suspension (500 mg nitazoxanide) twice daily for 3 consecutive days. Patients were instructed to take their medication with food and were given a diary with instructions to record administration of the medication, stool frequency and consistency, and other symptoms. In addition to the study medication, all patients received routine care including fluid replacement therapy and nutritional and metabolic management of diarrhea. The patients returned to the clinic on day 7 after initiation of treatment for a physical examination and evaluation of clinical response. Two stool samples collected at least 24 hours apart between days 7 and 10 and a third stool sample collected on day 14 were examined as described earlier. The day-14 stool examination was collected for potential scientific value but was not considered for evaluation of microbiologic response because of the potential for re-infection (the incubation period for Cryptosporidium...
Adverse events were recorded on the appropriate case report forms, and the severity of each adverse event was graded on a 4-point scale as follows: mild, moderate, severe, life-threatening. If applicable, adverse events were classified as serious or unexpected, and the relationship to the study drug was recorded.

Randomization

On enrollment, each patient sequentially was assigned a number corresponding to the number on his/her package of study medication. The computer-generated randomization list and the packaging of study medication were prepared by the study sponsor, Romark Laboratories. The patients, principal investigators, and their staffs and laboratory personnel were blinded to the treatment assignment for patients in the nitazoxanide or placebo tablet groups. Patients receiving nitazoxanide suspension and their physicians were aware that they were receiving active drug, but the laboratory personnel conducting stool examinations remained blinded as to their treatment assignment.

Statistical Analysis

The statistical analyses were conducted using JMP software version 5.1.1 (SAS Institute Inc., Cary, NC). The population used for efficacy analyses was defined prospectively as all patients randomized to the study excluding (1) patients with no Cryptosporidium oocysts in their baseline stool sample and (2) patients with other identified pathogens in the baseline stool sample. Patients who failed to complete the study were treated as failures. Proportional clinical and microbiologic response rates and the frequency of adverse events were compared by treatment group using 2-sided Fisher exact tests using an \( \alpha \) value of .05. A 95% confidence interval was calculated for the difference in proportional response rates reported for the 2 active treatment groups.

Results

Study Population

Ninety patients fulfilling the inclusion criteria were enrolled in the 2 studies between February 2003 and October 2004, and each of these 90 patients completed the study (Figure 1). Four patients were excluded from the efficacy analyses because they had no Cryptosporidium oocysts in their baseline stool sample. All 86 patients included in the efficacy analyses had Cryptosporidium identified as the sole cause of diarrhea. There were no other bacterial or parasitic pathogens identified in the stool samples of these patients at screening or at baseline.

The patients included in the efficacy analyses were well distributed among the active and placebo treatment groups with no differences in age, sex, stool frequency, stool consistency, duration of diarrhea, or physical examination abnormalities. Demographic and disease-related characteristics of the study population are summarized by treatment group in Table 1.

Other symptoms reported by the patients included abdominal pain/cramps (69%), mucus in stool (15%), nausea (7%), vomiting (3%), urgency tenesmus (3%), fever (2%), and abdominal distention (2%).

Efficacy

Clinical and microbiologic response rates are presented by treatment group in Table 2.

There was no significant difference between the response rates reported for the active tablets and active suspension groups. The difference in clinical response rates (active tablets minus active suspension) was +9% (95% confidence interval: +28% to −10%). The difference in microbiologic response rates (active tablets minus active suspension) was +3% (95% confidence interval: +21% to −17%).

Fifty-one patients were enrolled at the Benha site and 35 patients were enrolled at the Alexandria site. Proportional clinical and microbiologic response rates by treatment group were similar for the 2 study sites. Clinical response rates for the Benha site were 17 of 18 (94%), 6 of 17 (35%), and 14 of 16 (88%) for the active tablet, placebo tablet, and active suspension treatment groups compared with 10 of 10 (100%), 5 of 10 (50%), and 13 of 15 (87%), respectively, for the Alexandria site. Likewise, microbiologic response rates for the Benha site were 16 of 18 (89%), 5 of 17 (29%), and 15 of 16 (94%) for
the active tablet, placebo tablet, and active suspension treatment groups compared with 10 of 10 (100%), 5 of 10 (50%), and 13 of 15 (87%), respectively, for the Alexandria site.

To obtain longer follow-up data for the patients, the protocol was amended during the course of the study to require collection of physical examination and clinical response data at day 14. These data were collected for 40 patients. The proportion of patients with clinical responses that were considered well at day 14 were 12 of 12 (100%) for the active tablets, 15 of 17 (88%) for the suspension treatment group, and 4 of 11 (36%) for the placebo tablet group ($P < .0001$). All patients with well clinical responses at day 7 maintained their responses at day 14.

A secondary efficacy analysis was conducted for all patients randomized to the study. In this analysis, clinical response rates were 28 of 29 (97%), 14 of 30 (47%), and 27 of 31 (87%) for the active tablet, placebo tablet, and suspension treatment groups, respectively ($P < .0001$). Microbiologic response rates were 26 of 29 (90%), 13 of 30 (43%), and 28 of 31 (90%) for the active tablet, placebo tablet, and suspension treatment groups, respectively ($P < .0001$). In each case, the response rates remained significantly higher for each of the active treatment groups than for the placebo treatment group.

### Safety and Tolerability

During questioning at the follow-up evaluation, 16 patients reported 1 or more adverse events irrespective of causality. The adverse events consisted of fatigue (2 nitazoxanide tablets, 2 nitazoxanide suspension, 2 placebo), drowsiness (1 nitazoxanide tablets, 3 nitazoxanide suspension, 2 placebo), yellowish urine (1 nitazoxanide tablet, 1 placebo), abdominal pain (1 nitazoxanide tablet, 1 nitazoxanide suspension), headache (1 nitazoxanide suspension, 1 placebo), nausea (1 nitazoxanide suspension, 1 placebo), dyspepsia (1 nitazoxanide tablet, 1 nitazoxanide suspension), dysuria (1 nitazoxanide tablet), back pain (1 nitazoxanide tablet), and enlarged abdomen (1 nitazoxanide suspension). All of the adverse events were mild and transient in nature with none requiring discontinuation of treatment.

### Discussion

Persistent diarrhea and enteritis is a disease state associated with significant morbidity and economic costs (medical costs and lost productivity) in developed countries and longer-term consequences to health in developing countries. In this study we identified Cryptosporidium as a cause of persistent diarrhea and enteritis in nonimmunodeficient

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### Table 1. Demographic and Disease-Related Characteristics of Evaluable Patients at Baseline

<table>
<thead>
<tr>
<th></th>
<th>Active tablet (n = 28)</th>
<th>Placebo tablet (n = 27)</th>
<th>Active suspension (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male/Female (n)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>14/14</td>
<td>14/13</td>
<td>12/19</td>
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<tr>
<td>Age, y</td>
<td>Mean ± SD</td>
<td>Range</td>
<td></td>
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<tr>
<td></td>
<td>36 ± 17</td>
<td>12–67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27 ± 15</td>
<td>12–55</td>
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<td></td>
<td>30 ± 15</td>
<td>12–59</td>
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<tr>
<td>Weight, kg</td>
<td>Mean</td>
<td>Range</td>
<td></td>
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<tr>
<td></td>
<td>67 ± 20</td>
<td>26–109</td>
<td></td>
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<tr>
<td></td>
<td>62 ± 19</td>
<td>30–105</td>
<td></td>
</tr>
<tr>
<td></td>
<td>66 ± 16</td>
<td>25–100</td>
<td></td>
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<tr>
<td>Duration of diarrhea, d</td>
<td>Mean</td>
<td>Range</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 ± 11</td>
<td>4–58</td>
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<tr>
<td></td>
<td>8 ± 2</td>
<td>6–12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 ± 17</td>
<td>4–100</td>
<td></td>
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<tr>
<td>Stool frequency, n</td>
<td>Liquid/soft</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18/10</td>
<td>20/7</td>
<td>23/8</td>
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<tr>
<td></td>
<td>17/10/1</td>
<td>12/15/0</td>
<td>15/4/2</td>
</tr>
</tbody>
</table>

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### Table 2. Response Rates by Treatment Group at Study Day 7

<table>
<thead>
<tr>
<th></th>
<th>Active tablets</th>
<th>Placebo tablets</th>
<th>Active suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of patients with well response</td>
<td>27/28 (96%)$^a$</td>
<td>11/27 (41%)</td>
<td>27/31 (87%)$^p$</td>
</tr>
<tr>
<td>Proportion of patients with no Cryptosporidium oocysts detected in posttreatment stool samples$^c$</td>
<td>26/28 (93%)$^a$</td>
<td>10/27 (37%)</td>
<td>28/31 (90%)$^a$</td>
</tr>
</tbody>
</table>

$^aP < .0001$ compared with placebo response rates.
$^pP = .0003$ compared with placebo response rate.
$^c$All patients submitted 2 stool samples between study days 7 and 10 except for 1 patient in the active suspension group. This patient was treated as a failure in the efficacy analysis according to the protocol.
adults and showed that this disease can be treated effectively with a 3-day course of nitazoxanide. The patients enrolled in this study presented with diarrhea and other symptoms (principally abdominal pain/cramps) of 4 to 100 days' duration. Ninety-six percent of patients treated with the nitazoxanide tablets responded to treatment with complete resolution of symptoms by study day 7 compared with 41% of patients receiving the placebo tablet (P < .0001). Patients randomized to the placebo treatment group who did not respond by study day 7 remained symptomatic at study day 14, which is indicative of the persistent and possibly chronic nature of the disease. Clinical responses were correlated closely to the absence of Cryptosporidium oocysts in posttreatment stool samples.

The disease patterns shown by patients enrolled in this study are similar to those reported for patients in the United States. In an outbreak setting in the United States, the duration of symptomatic cryptosporidiosis commonly is reported to be up to 4 to 8 weeks, with relapsing episodes commonly reported. Other investigators have described illness associated with Cryptosporidium infection as "persistent relapsing diarrhea." We did not address the potential for long-term effects of Cryptosporidium infection in our study population. Given that Cryptosporidium is an intracellular protozoan infecting the gastrointestinal epithelium and that infection often lasts for several weeks or months, it is reasonable to hypothesize that Cryptosporidium infection could be associated with postinfectious irritable syndrome. Cryptosporidium infection has been associated with long-term effects on physical fitness and cognitive development in young children with cryptosporidiosis during early childhood. Further study of the potential for long-term effects of Cryptosporidium infection in adults may be warranted.

In conclusion, the present study showed the effectiveness of nitazoxanide in treating diarrhea and enteritis associated with Cryptosporidium species in nonimmunodeficient adults and pediatric patients at least 12 years of age. A 3-day course of treatment significantly reduced the duration of symptoms and oocyst excretion.

References

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Jean-François Rossignol is an employee of and owns an equity interest in Romark Laboratories, LC, the pharmaceutical company that owns the patent rights related to nitazoxanide. The study was supported by a grant from Romark Laboratories, LC, Tampa, Florida.