Treatment of Diarrhea Caused by Cryptosporidium parvum: A Prospective Randomized, Double-Blind, Placebo-Controlled Study of Nitazoxanide

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A prospective randomized, double-blind, placebo-controlled study was conducted in 50 adults and 50 children from the Nile delta of Egypt, to evaluate the efficacy of nitazoxanide in treating diarrhea caused by Cryptosporidium parvum. Nitazoxanide was administered in 500-mg doses twice daily for 3 days in adults and adolescents, in 200-mg doses twice daily for 3 days in children aged 4–11 years, and in 100-mg doses twice daily for 3 days in children aged 1–3 years. At 7 days after initiation of therapy, diarrhea had resolved in 39 (80%) of the 49 patients in the nitazoxanide treatment group, compared with 20 (41%) in the placebo group (P < .0001). Diarrhea was resolved in most patients receiving nitazoxanide within 3 or 4 days of treatment initiation. Nitazoxanide treatment reduced the duration of both diarrhea (P < .0001) and oocyst shedding (P < .0001).

Cryptosporidium parvum is an important cause of diarrheal disease in children and adults worldwide [1, 2]. Although many antimicrobial drugs are used to treat C. parvum infections, none has proved effective in treating the disease [3]. Nitazoxanide, a nitrothiazolyl-salicylamide derivative [4], has shown activity against C. parvum in cell culture and in animal models [5–7]. In humans, a 3-day course of nitazoxanide is effective in treating enteric protozoan infections caused by Giardia intestinalis, Entamoeba histolytica and E. dispar, Blastocystis hominis, Balantium coli, Isospora belli, and Cyclospora cayetanensis [8–10]. It is also effective in treating diarrhea caused by C. parvum in patients with AIDS; its activity varies, depending on the degree of immunosuppression and the duration of treatment [11, 12]. Here we report the use of nitazoxanide in the treatment of diarrhea caused by C. parvum in immunocompetent adults and children.

Subjects and Methods

Study design. This was a prospective randomized, double-blind, placebo-controlled study. In the absence of guidelines for the evaluation of a new drug for treating cryptosporidiosis, published guidelines for evaluation of new anti-infective drugs for treating diarrhea caused by other enteric protozoa, G. intestinalis and E. histolytica [13, 14], were consulted in designing the study. Study enrollment was stratified to include 50 adults and adolescents (aged 12–65 years) and 50 children (aged 1–11 years). In addition to the blinded study medication, all patients received routine care, including fluid replacement therapy (oral or intravenous) and nutritional and metabolic management of diarrhea. The primary study end points were clinical response, recorded at the day 7 follow-up visit, and parasitologic response, recorded on the basis of examination of 2 stool samples collected between days 7 and 10 after initiation of treatment. The time from initiation of treatment (administration of the first dose) to passage of the last unformed stool (reported by the patient at the follow-up visit) was evaluated as a secondary end point. Patients, personnel assessing clinical response, and laboratory personnel evaluating the stool samples were blinded, so that critical data for each end point (date of last unformed stool, clinical response at day 7, results of posttreatment stool examinations, and adverse events) were generated without knowledge of treatment assignment.

Study medication. Adults and adolescents (aged 12–65 years) received 1 500-mg yellow film-coated nitazoxanide tablet or a matching placebo tablet twice daily for 3 consecutive days. Children received 5 mL (ages 1–3 years) or 10 mL (ages 4–11 years) of nitazoxanide (100 mg/5 mL of strawberry-flavored oral suspension) or a matching placebo twice daily for 3 consecutive days. Patients were instructed to take the medication with food. The pharmaceutical formulations of nitazoxanide and the matching placebo were supplied by Romark Laboratories.

Subjects. Children, adults, and adolescents presenting with diarrhea at the outpatient clinic of the Department of Hepatology,
Gastroenterology, and Infectious Diseases of the Benha University Hospital in the Nile delta of Egypt were screened for study enrollment. Patients with diarrhea and with C. parvum oocysts in a stool sample within 7 days before enrollment were eligible for enrollment. Diarrhea was defined as >4 unformed stools per day. Stools were considered to be unformed if they were soft (taking the shape of the container) or watery (could be poured or soaked into a diaper). Children <1 year old, adults >65 years old, pregnant women, patients using any drug with antiprotozoal activity within 2 weeks of enrollment, and patients known to have or suspected of having AIDS were excluded from the study.

Assessment of cause of diarrhea. Fecal samples underwent a direct examination, an examination after concentration, Ziehl-Neelsen staining, and an immunofluorescence assay (MeriFluor; Meridian Diagnostics), for assessment of parasitic causes of diarrhea, including adherent or toxigenic Escherichia coli. Because of the time required for the stool culture, patients who otherwise satisfied the criteria for inclusion were enrolled in the study before the results of the stool culture were known; patients with a positive stool culture for bacterial causes of diarrhea were excluded from the analyses of clinical response.

Study procedures and follow-up. 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, and presence of mucus or blood). Patients were given instructions for taking their study medication, a follow-up visit was scheduled, and, in the event that diarrhea resolved before the follow-up visit, they were asked to record the date of their last unformed stool.

Patients returned to the clinic on day 7 (±2 days) after the initiation of treatment for a physical examination. At that time they were evaluated for clinical improvement; if their diarrhea had resolved, they reported the date of their last unformed stool. Each patient’s clinical response was assigned as either “well” (no symptoms, no watery stools, ≤2 soft stools and no hematochezia within the past 24 h, or no symptoms and no unformed stools within the past 48 h) or “continuing illness” (any number of watery stools, ≥2 soft stools per 24 h, or hematochezia or enteric symptoms plus the passage of any number of soft or watery stools during the past 48 h). Two stool samples collected at least 24-h apart between days 7 and 10 were examined for the presence of C. parvum oocysts. Each patient’s parasitologic response was recorded as either “eradicated” (no oocysts observed in either posttreatment stool sample) or “persistance” (oocysts observed in either or both posttreatment stool samples). Adverse events were recorded on the appropriate case report forms, and the severity of each adverse event was graded on a 4-point scale: mild, moderate, severe, or life threatening. When applicable, adverse events were classified as serious or unexpected, and the relationship to the study drug was recorded.

Statistical analysis. Statistical analyses were done with JMP software version 3.2 (SAS Institute). The proportional clinical and parasitologic response rates and the frequency of adverse events were compared by treatment group by χ² or Fisher’s exact tests. The times from initiation of treatment to passage of the last unformed stool were compared by treatment group, using a Kaplan-Meier survival test.

Results

Study population. Fecal samples of 725 diarrheic patients were screened to identify and enroll 50 adults and adolescents (>11 years old) and 50 children (<11 years old) in the study. Among the 50 adults and adolescents, there were 27 male and 23 female subjects (age range, 15–62 years; mean ± SD, 35.22 ± 12.41 years). Of the 50 children, 27 (17 boys and 10 girls) were 4–11 years old (mean ± SD, 7.69 ± 2.14 years), and 23 (12 boys and 11 girls) were 1–3 years old (mean ± SD, 2.22 ± 0.78 years).

At enrollment, 96 of the 100 patients passed 5–10 stools per day, and 4 passed >10 stools per day (3 of the 4 were 1–3 years old). Thirty-nine patients had liquid stools, and 61 reported semisolid stools. Younger patients were more likely to have liquid stools (16/23, ages 1–3 years; 12/27, ages 4–11 years; 11/50 adults and adolescents; P = .0004). The mean duration of diarrhea at enrollment was 13.22 days (range, 5–97 days; median, 9 days) for patients in the nitazoxanide treatment group, compared with 12.76 days (range, 5–90 days; median, 9.5 days) for the placebo group. Other symptoms associated with the patients’ disease included abdominal pain, colic, cramps, or distention (87 subjects); vomiting (3 subjects); fever (11 subjects); dehydration (8 subjects); and backache (2 subjects). The patients enrolled in the study were well distributed among the active and placebo treatment groups, with no differences in age, sex, stool frequency, stool consistency, duration of diarrhea, or abnormalities found during physical examination.

Of the 100 study subjects, 99 completed the study (50 adults and adolescents and 49 children). One child randomized to the nitazoxanide treatment group withdrew before taking any study medication. Another child randomized to the placebo group was excluded from the analysis of clinical response because he had a positive stool culture for Salmonella species at baseline. Three adults, one in the nitazoxanide group and 2 in the placebo group, missed 2–4 doses of the study medication but returned for follow-up.

Efficacy. Table 1 shows clinical and parasitologic response rates by treatment group. Of 49 patients randomized to the nitazoxanide treatment group, 39 (80%) showed a “well” clinical response at the day 7 follow-up visit, compared with 20 (41%) of 49 in the placebo group (P < .0001), and 33 (67%) of 49 patients in the nitazoxanide group had no oocysts in either of the 2 posttreatment stool samples, compared with 11 (22%) of 50 in the placebo group (P < .0001). Significant statistical differences were also observed for subgroups of children and adults. Although the response rates for nitazoxanide appeared to be higher in children than in adults, this was not statistically significant.

The effect of nitazoxanide is further illustrated in figure 1, which presents a Kaplan-Meier analysis of diarrhea survival time (time from initiation of treatment to passage of the last unformed stool). The median time from initiation of therapy to passage of the last unformed stool was 3 days in the nitazoxanide group, whereas a median time could not be calculated...
Table 1. Proportions of patients in whom diarrhea was resolved and in whom no oocysts were detected in posttreatment stool samples, by treatment group.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Nitazoxanide(^a)</th>
<th>Placebo(^a)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients in whom diarrhea was resolved</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients (adults, adolescents, and children)</td>
<td>39/49 (80)</td>
<td>20/49 (41)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Adults and adolescents only</td>
<td>18/25 (72)</td>
<td>11/25 (44)</td>
<td>.0845</td>
</tr>
<tr>
<td>Children only</td>
<td>21/24 (88)</td>
<td>9/24 (38)</td>
<td>.0004</td>
</tr>
<tr>
<td>Patients with no oocysts detected in posttreatment stool samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients (adults, adolescents, and children)</td>
<td>33/49 (67)</td>
<td>11/50 (22)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Adults and adolescents only</td>
<td>15/25 (60)</td>
<td>6/25 (24)</td>
<td>.0209</td>
</tr>
<tr>
<td>Children only</td>
<td>18/24 (75)</td>
<td>5/25 (20)</td>
<td>.0002</td>
</tr>
</tbody>
</table>

\(^a\) Data are no. responding/no. tested (%).
\(^b\) Fisher’s exact test, 2-sided.

for the placebo group, because 59% of the patients still had diarrhea at the end of the follow-up period.

Eighteen patients (11 children and 7 adolescents and adults) were positive for another protozoan parasite in stool at study enrollment. These included *Giardia lamblia* \((n = 9)\), *E. histolytica* \((n = 6)\), and *B. hominis* \((n = 3)\). Eleven were randomized to the nitazoxanide treatment group and 7 to the placebo group. A separate analysis was done of the subset of 82 patients for whom *C. parvum* was the only potential cause of diarrhea. In this analysis, the results were almost identical to those shown in table 1 (overall clinical response rate of 80% for nitazoxanide vs. 35% for the placebo, \(P < .0001\); parasitologic response rate of 67% for nitazoxanide vs. 23% for placebo, \(P = .0001\)).

**Safety and tolerance.** Twelve adverse events were reported by 11 patients (4 children and 7 adolescents or adults) in the nitazoxanide treatment group, compared with 14 adverse events reported by 13 patients (7 children and 6 adolescents or adults) in the placebo group. The adverse events consisted of abdominal pain (3 placebo and 1 nitazoxanide), dyspepsia (2 placebo), worsening diarrhea (2 placebo and 1 nitazoxanide), constipation (1 nitazoxanide), anorexia (1 placebo and 1 nitazoxanide), yellow discoloration of urine (1 placebo and 3 nitazoxanide), dysuria (1 placebo), dizziness (2 placebo and 2 nitazoxanide), drowsiness (1 placebo and 2 nitazoxanide), dry mouth (1 nitazoxanide), and facial edema (1 placebo). All adverse events were mild and transient in nature. Two adverse events, both episodes of dizziness in adult patients (1 receiving placebo and 1 receiving active drug) resulted in discontinuation of therapy.

**Discussion**

*Cryptosporidium* infection is underdiagnosed, because oocysts may be shed sporadically and because specialized diagnostic techniques are necessary for detection [1]. Nevertheless, reports in recent years have clearly established the importance of *Cryptosporidium* infection as a significant cause of diarrhea that can be persistent and can be associated with long-term effects on growth and development [15]. The availability of an effective treatment might alleviate the requirement for hospitalization due to acute illness and might limit potential long-term consequences of the disease.

The present study demonstrated the activity of nitazoxanide in treating diarrhea caused by *C. parvum* in an immunocompetent population. A 3-day course of treatment reduced the duration of diarrhea \((P < .0001)\) and of oocyst excretion \((P < .0001)\). The safety and tolerance of nitazoxanide also were well documented in children, adolescents, and adults. Adverse events reported by patients in the nitazoxanide treatment group were substantially identical to those reported by patients receiving placebo.

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**Figure 1.** Proportion (0.0–1.0) of patients experiencing diarrhea (unformed stools) on days 1–15 after initiation of treatment, by Kaplan-Meier analysis of diarrhea survival time \((P = .0003, \text{log-rank test; } \chi^2 = 12.9926, df = 1)\).
References


