Effect of Nitazoxanide in Persistent Diarrhea and Enteritis Associated With *Blastocystis hominis*

JEAN–FRANÇOIS ROSSIGNOL,* SAMIR M. KABIL,† MOHAMMED SAID,† HATEM SAMIR,† and AZZA M. YOUNIS§

*The Romark Institute for Medical Research, Tampa, Florida; †Department of Hepatology, Gastroenterology and Infectious Diseases, University of Zagazig, Benha Faculty of Medicine, Benha, Egypt; and the §Department of Parasitology, University of Cairo, Kasr El-Ainy, Faculty of Medicine, Cairo, Egypt

Background & Aims: The aim of this study was to evaluate the efficacy of nitazoxanide for the treatment of diarrhea and enteritis associated with *Blastocystis hominis* as the sole identified pathogen in children and adults from the Nile delta of Egypt. **Methods:** Two prospective, randomized, double-blind, placebo-controlled studies were conducted. Nitazoxanide 500 mg (as a 500-mg tablet) was administered twice daily for 3 days in patients aged 12 years or older, 200 mg (as 10 mL of an oral suspension) was administered twice daily for 3 days in patients aged 4–11 years, and 100 mg (as 5 mL of an oral suspension) was administered twice daily for 3 days in patients aged 1–3 years. **Results:** Four days after the completion of therapy, 36 (86%) of the 42 patients who received nitazoxanide showed resolution of symptoms compared with 16 (38%) of 42 patients who received placebo ($P < .0001$). Thirty-six (86%) of the 42 patients who received nitazoxanide were free of *B hominis* organisms in each of 3 posttreatment stool samples compared with only 5 (12%) of 42 patients who received placebo ($P < .0001$). Response rates in patients receiving the tablets and the suspension were identical. **Conclusions:** These findings suggest that *B hominis* is pathogenic in some patients and can be treated effectively with nitazoxanide. Alternatively, the possibility that nitazoxanide is effective in treating other unidentified causes of persistent diarrhea and enteritis warrants further study.

Blastocystosis is caused by *Blastocystis hominis*, a parasite that commonly resides in the cecum and large bowel of human beings. The pathogenicity of *B hominis* has been an issue of controversy. Initially, it was believed to be commensal, however, recent information from retrospective studies and experimental models in animals support its pathogenicity.[1–4]

Blastocystosis has been characterized by symptoms including diarrhea, abdominal pain, nausea, vomiting, flatulence, anorexia, and malaise,[1–4] and has been implicated in patients diagnosed with irritable bowel syndrome.[5] Data on treatment of blastocystosis are limited primarily to anecdotal studies in which metronidazole and iodoquinol, the most commonly recommended therapies, were used showing variable results.[2] A recent, single-blind, placebo-controlled study of metronidazole administered 1.5 g/day for 10 consecutive days showed improvement of diarrhea in the metronidazole-treated group and a higher rate of parasite clearance.[6]

Nitazoxanide (Alinia; Romark Pharmaceuticals, Tampa, FL) is indicated in the United States for treatment of persistent diarrhea caused by *Giardia* and *Cryptosporidium* and is being investigated for treatment of diarrhea caused by enteroviruses, *Clostridium difficile*–associated diarrhea, and Crohn's disease. It is active against a broad range of enteropathogens including protozoa, bacteria, and viruses by 3 mechanisms: interference with energy metabolism in anaerobic organisms (protozoa and bacteria), inhibition of transcription/replication in infected cells (viruses), and inhibition of secretion of proinflammatory cytokines.

Double-blind, placebo-controlled clinical studies have shown that a 3-day course of nitazoxanide is effective in treating diarrhea and enteritis caused by *Cryptosporidium* species, *Giardia intestinalis*, and *Entamoeba histolytica*, and in eliminating the organisms from the stool.[7–9] The same course of treatment also has been reported to be effective in treating enteric infections caused by *B hominis*, *Balantidium coli*, and *Cyclospora cayetanensis*.[10–12]

We report a prospective, randomized, double-blind, placebo-controlled clinical study of nitazoxanide in treating persistent diarrhea associated with blastocystosis.

Patients and Methods

Study Design

We performed 2 prospective, randomized, double-blind, placebo-controlled studies, one in patients aged 12 years and older using nitazoxanide tablets and another in children

1542-3565/05/$30.00
PII: 10.1053/S1542-3565(05)00427-1

aged 1–11 years using nitazoxanide oral suspension, to evaluate the effectiveness of nitazoxanide in treating persistent diarrhea and enteritis associated with *B hominis* as the sole identified pathogen. In the absence of guidelines for the evaluation of a new drug for treating blastocystosis, published guidelines for evaluation of new anti-infective drugs for treating diarrhea caused by *G intestinalis* were consulted in designing the study.[13] The primary end point of the study was clinical response recorded at the day 7 follow-up visit. Clinical response was defined as either *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 as *continuing illness*. Microbiologic response, defined as either *eradicated* (no *B hominis* organisms observed in either of 2 stool samples collected between study days 7 and 10) or *persistence*, was evaluated as a secondary end point. Each study was designed to enroll 50 patients. Previous studies of nitazoxanide in treating diarrhea caused by 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 35%. By using these assumed response rates, a sample size of 50 patients (25 patients per treatment group) was deemed sufficiently powerful (88%) to show that treatment with nitazoxanide is superior to treatment with placebo using a 2-sided Fisher exact test and a 5% significance level.

Patients

Patients 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 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 *C parvum*, *G intestinalis*, and *E histolytica*. Before screening, written informed consent was obtained from each of the adult patients, and in the case of children consent was obtained from their parents or guardians. If possible, written informed consent also was obtained from the children. Patients with persistent diarrhea (≥3 bowel movements/day) and 1 or more enteric symptoms (eg, abdominal pain, nausea, vomiting, or flatulence) and with *B hominis* organisms 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, and presence of mucus or blood). Patients 12 years of age and older received 1 nitazoxanide 500-mg tablet or a matching placebo tablet twice daily for 3 consecutive days. Patients 4–11 years of age received 10 mL of nitazoxanide 100 mg/5 mL suspension or a matching placebo twice daily for 3 consecutive days. Patients 1–3 years of age received 5 mL of nitazoxanide 100 mg/5 mL suspension or a matching placebo twice daily for 3 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 blinded 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 subjected to microbiologic examination. The day 14 stool examination was conducted for its scientific value, but the day 14 results were not considered as part of the definition of microbiologic response (prospectively defined) because of the potential for re-infection. 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, 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 was assigned a number sequentially that corresponded to the number on his/her bottle of study medication. The computer-generated randomization list and the packaging of study medications were prepared by the study sponsor, Romark Laboratories. The patients, principal investigators, and their staffs and laboratory personnel were blinded so that critical data for each of the end points (clinical response, results of posttreatment stool examinations, and adverse events) were generated without knowledge of 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 *B hominis* organisms in their baseline stool sample and (2) patients with other identified pathogens in the baseline

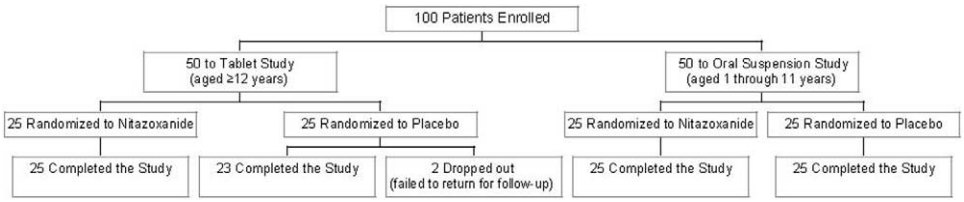

Figure 1. Patient disposition flow chart.

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 with an α of .05.

Results

Study Population

One hundred patients fulfilling the inclusion criteria were enrolled in the 2 studies between March 16, 2004, and June 6, 2004. Fifty adults and adolescents (age, ≥12 y) were enrolled in the tablet study, and 50 children (age, 1–11 y) were enrolled in the suspension study. Sixteen patients were excluded from the efficacy analyses because of the results of the baseline stool examination: 6 were negative for *B hominis*, 7 were positive for *G intestinalis*, 2 were positive for *E histolytica*, and 1 was positive for *Cryptosporidium* species. Two patients in the placebo tablet group failed to return for follow-up evaluation and were considered treatment failures according to the protocol (Figure 1).

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.

Efficacy

Clinical and microbiologic response rates are presented by treatment group in Table 2. The correlation of clinical response to microbiologic response was near perfect in the nitazoxanide groups with 35 of the 36 clinical responders being free of *B hominis* organisms in the day 7–10 stool samples. Each of the 26 clinical failures in the placebo groups had *B hominis* organisms in their fol-

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

	Tablet study		Suspension study	
	Active (n = 21)	Placebo (n = 21)	Active (n = 21)	Placebo (n = 21)
Sex				
Male/Female (n)	10/11	6/15	11/10	10/11
Age (*y*)				
Mean ± SD	23 ± 12	20 ± 10	8 ± 3	8 ± 2
Range	12–43	12–39	2–11	3–11
Weight (*kg*)				
Mean	56 ± 19	54 ± 19	27 ± 9	28 ± 8
Range	27–81	30–88	14–51	15–43
Duration of diarrhea (*days*)				
Mean	8 ± 4	7 ± 2	6 ± 1	7 ± 2
Range	4–22	4–10	3–9	4–9
Stool frequency (n)				
3–4 per day/5–10 per day	16/5	16/5	18/3	18/3
Stool consistency (n)				
Liquid/soft	6/15	4/17	11/10	8/13
Other symptoms (n)				
Abdominal pain/cramps	19	20	20	19
Fever	2	4	3	3
Mucus in stool	2	4	3	3
Nausea	3	1	3	—
Flatulence	2	3	1	1
Blood in stool	4	1	—	1
Vomiting	1	1	3	—
Anorexia	—	—	1	3

Table 2. Response Rates by Treatment Group

	Nitazoxanide	Placebo	P^a
Clinical response rate[b]	36/42 (86%)	16/42 (38%)	<.0001
Microbiologic response rate[c]	36/42 (86%)	5/42 (12%)	<.0001

[a]Fisher exact test 2-sided.
[b]Proportion of patients resolving symptoms. Clinical response rates for each study were identical: 18 of 21 (86%) for the nitazoxanide treatment group compared with 5 of 21 (38%) for the placebo group ($P = .0036$).
[c]Proportion of patients with no *B hominis* organisms detected in posttreatment stool samples. Microbiologic response rates for the tablet study were 18 of 21 (86%) for the nitazoxanide treatment group compared with 3 of 21 (14%) for the placebo group ($P < .0001$). Microbiologic response rates for the suspension study were 18 of 21 (86%) for the nitazoxanide treatment group compared with 2 of 21 (10%) for the placebo group ($P < .0001$).

low-up stool samples, and only 5 of the 16 clinical responders in the placebo groups were negative for the parasite at the day 7–10 follow-up evaluation. The stool samples collected at day 14 were negative for all nitazoxanide-treated patients and for 12 of the 21 patients in the placebo group.

Safety and Tolerability

On questioning at follow-up evaluation, 30 patients reported 1 or more adverse events irrespective of causality. The adverse events consisted of fatigue (7 nitazoxanide, 7 placebo), drowsiness (7 nitazoxanide, 5 placebo), yellowish urine (6 nitazoxanide, 1 placebo), abdominal pain (5 nitazoxanide), headache (2 nitazoxanide), nausea (1 nitazoxanide, 1 placebo), and vomiting (1 placebo). Each 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.[14]

In recent years, enteric protozoan infections, particularly *Giardia* and *Cryptosporidium*, have been recognized as common causes of persistent diarrhea and enteritis. *B hominis* is another enteric parasite that is recognized worldwide, some reports suggesting that it may be as common as *Giardia* and *Cryptosporidium*.[4,15,16]

The pathogenicity of *B hominis* has been the subject of debate because many patients excreting *B hominis* organisms are asymptomatic.[1–4] With reports of animal models showing pathogenicity, retrospective studies of the association of symptoms with *B hominis* infection, and anecdotal case reports, the case for the pathogenicity of *B hominis* has become more robust.[1–4] Differences among isolates of *B hominis* have been proposed as an explanation for the lack of symptoms in some patients carrying *B hominis* infection.[17]

A recent study associated *B hominis* infection with irritable bowel syndrome. In that study, 46% of 95 irritable bowel syndrome patients were stool positive for *B hominis* compared with only 7% of 55 controls ($P < .001$).[18]

Here we identified patients with persistent diarrhea and enteric symptoms associated with *B hominis* as the sole identified pathogen and showed that they responded to a 3-day course of nitazoxanide. Patients enrolled in this study reported having symptoms for approximately 7 days at the time of enrollment, and 62% of the placebo patients still were symptomatic on study day 7. This is consistent with previous reports suggesting that symptoms last more than 1 month in immunocompetent children and adults with *Blastocystis* infection who do not receive pharmacotherapy.[6,19]

Blastocystis infection was found in patients of all ages, and nitazoxanide therapy was equally effective in both children and adults. The dose of nitazoxanide and duration of therapy is much less than that shown to be effective for metronidazole,[6] a fact that may be attributed to higher concentrations of drug in the lower gastrointestinal tract. Although nitazoxanide is absorbed and available in the plasma and tissues after oral administration, its active metabolites are excreted in bile, and two thirds of an oral dose passes through the colon and is excreted in stool.[20]

Blastocystis has been reported to be prevalent particularly in immunosuppressed patients,[21] but patients enrolled in the present study were immunocompetent. It is possible that a longer course of treatment with nitazoxanide may be required in patients with compromised immune systems as has been reported with *Cryptosporidium*, *Giardia*, and *Enterocytozoon bieneusi* infection.[22–25]

Although we cannot exclude the possibility of another undetected cause of diarrhea and enteritis against which nitazoxanide might be effective, the patients in the present studies submitted 5 stool samples over approximately 3 weeks, and the data did not suggest the presence of other infections. Thus, the findings of this study support the growing body of evidence suggesting that *B hominis* is a human pathogen.

If, alternatively, *B hominis* is not a human pathogen, our findings would suggest that nitazoxanide is effective in resolving persistent diarrhea and enteritis of unknown

origin. This possibility deserves consideration and further study given the broad-spectrum activity of nitazoxanide against gastrointestinal pathogens and the frequent lack of sensitivity of stool examinations and cultures.

The present study showed the effectiveness of nitazoxanide in treating diarrhea and enteritis associated with *B hominis* as the sole identified pathogen. A 3-day course of treatment reduced the duration of symptoms ($P < .0001$) and organism excretion ($P < .0001$). Additional studies are warranted to evaluate fully the role of nitazoxanide as pharmacotherapy for persistent diarrhea and enteritis associated with *B hominis* and/or other enteric pathogens.

References

1. Petri WA Jr, Singh U, Ravdin JI. Enteric amebiasis. In: Guerrant RL, Walker DH, Weller PF, eds. Tropical infectious diseases: principles, pathogens & practice. Volume 1. Philadelphia: Churchill Livingstone, 1999:685–702.
2. Keystone JS, Kozarsky P. Isospora belli, Sarcocystis species, Blastocystis hominis, and Cyclospora. In: Mandell LM, Bennett JE, Dolin R, eds. Mandell, Douglas & Bennett's principles and practice of infectious diseases. Volume II. Philadelphia: Churchill Livingstone, 2000:2915–2920.
3. Stenzel DJ, Boreham PFL. *Blastocystis hominis* revisited. Clin Microbiol Rev 1996;9:563–584.
4. Leelayoova S, Rangsing R, Taamasri P, et al. Evidence of waterborne transmission of *Blastocystis hominis*. Am J Trop Med Hyg 2004;70:658–662.
5. Giacometti A, Cirioni O, Fiorentini A, et al. Irritable bowel syndrome in patients with *Blastocystis hominis* infection. Eur J Clin Microbiol Infect Dis 1999;18:436–439.
6. Nigro L, Larocca L, Massarelli L, et al. A placebo-controlled treatment trial of *Blastocystis hominis* infection with metronidazole. J Travel Med 2003;10:128–130.
7. Rossignol J-FA, Ayoub A, Ayers MS. Treatment of diarrhea caused by *Cryptosporidium parvum*: a prospective, randomized, double-blind placebo controlled study of nitazoxanide. J Infect Dis 2001; 184:103–106.
8. Rossignol JF, Ayoub A, Ayers MS. Treatment of diarrhea caused by *Giardia intestinalis* and *Entamoeba histolytica* or *E. dispar:* a double blind placebo controlled study of nitazoxanide. J Infect Dis 2001;184:381–384.
9. Amadi B, Mwiya M, Musuku J, et al. Effect of nitazoxanide on morbidity and mortality in Zambian children with cryptosporidiosis: a randomised controlled trial. Lancet 2002;360:1375–1380.
10. Vdovenko A, Williams J. *Blastocystis hominis*: neutral red supravital staining and its application to in vitro drug sensitivity testing. Parasit Res 2000;86:573–581.
11. Cimerman S, Ladeira MCT, Iuliano WA. Blastocystosis: nitazoxanide as a new therapeutic option. Rev Soc Bras Med Trop 2003;36:415–417.
12. Gilles HM, Hoffman PS. Treatment of intestinal parasitic infections, a review of nitazoxanide. Trends Parasitol 2002;18:95–97.
13. Cooperstock M, DuPont HL, Corrado ML, et al. Evaluation of new anti-infective drugs for the treatment of diarrhea caused by *Giardia lamblia*. Clin Infect Dis 1992;15(Suppl 1):S244–S248.
14. Guerrant RL, Van Gilder T, Steiner TS, et al. Practice guidelines for the management of infectious diarrhea. Clin Infect Dis 2001;32:331–350.
15. Kappus KD, Lundgren RG Jr, Juranek DD, et al. Intestinal parasitism in the United States: update on a continuing problem. Am J Trop Med Hyg 1994;50:705–713.
16. Amin OM. Seasonal prevalence of intestinal parasites in the United States during 2000. Am J Trop Med Hyg 2002;66:799–803.
17. Clark GC. Extensive genetic diversity in *Blastocystis hominis*. Mol Biochem Parasitol 1997;87:79–83.
18. Yakoob J, Jafri W, Jafri N, et al. Irritable bowel syndrome: in search of an etiology: role of Blastocystis hominis. Am J Trop Med Hyg 2004;70:383–385.
19. O'Gorman MA, Orenstein SR, Proujansky R, et al. Prevalence and characteristics of *Blastocystis hominis* infection in children. Clin Pediatr (Phila) 1993;32:91–96.
20. Broekhuysen J, Stockis A, Lins RL, et al. Nitazoxanide: pharmacokinetics and metabolism in man. Int J Clin Pharmacol Ther 2000;38:387–394.
21. Cirioni O, Giacometti A, Drenaggi D, Ancarani F, Scalise G. Prevalence and clinical relevance of *Blastocystis hominis* in diverse patient cohorts. Eur J Epidemiol 1999;15:389–393.
22. de la Tribonniére X, Valette M, Alfandari S. Oral nitazoxanide and paromomycin inhalation for systemic cryptosporidiosis in a patient with AIDS. Infection 1999;27:232.
23. Abboud P, Lemée V, Gargala G, et al. Successful treatment of metronidazole- and albendazole-resistant giardiasis with nitazoxanide in a patient with acquired immunodeficiency syndrome. Clin Infect Dis 2001;32:1792–1794.
24. Bicart-sée A, Massip P, Linas M-D, et al. Successful treatment with nitazoxanide of *Enterocytozoon bieneusi* microsporidiosis in a patient with AIDS. Antimicrob Agents Chemother 2000;44:167–168.
25. Rossignol J-F, Hidalgo H, Feregrino M, et al. A 'double-blind' placebo-controlled study of nitazoxanide in the treatment of cryptosporidial diarrhoea in AIDS patients in Mexico. Trans R Soc Trop Med Hyg 1998;92:663–666.

Address requests for reprints to: Jean-François Rossignol, The Romark Institute for Medical Research, 3000 Bayport Drive, Suite 200, Tampa, Florida 33607. e-mail: jrossignol@romark.com; fax: (813) 282-4910.

Supported by a grant from Romark Laboratories, LC, Tampa, FL. Dr 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.