Albendazole for Treatment and Prophylaxis of Microsporidiosis Due to *Encephalitozoon intestinalis* in Patients with AIDS: A Randomized Double-Blind Controlled Trial

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A double-blind placebo-controlled trial was conducted to assess the efficacy and safety of albendazole (400 mg twice daily for 3 weeks) for the treatment of *Encephalitozoon intestinalis* infection in patients with AIDS. Clearance of microsporidia from the intestinal tract was obtained in 4 of 4 patients in the albendazole group versus 0 of 4 in the control group (*P* = .01, one-sided Fisher’s exact test) and was associated with significant clinical benefit. All 4 controls subsequently cleared microsporidia following open-labeled albendazole treatment. To investigate the effect of albendazole in preventing relapse, these 8 patients were then randomly assigned to receive either albendazole (400 mg twice daily) or no treatment for the next 12 months. Albendazole significantly delayed the occurrence of relapse (*P* = .04, one-sided log-rank test). In human immunodeficiency virus–infected patients with *E. intestinalis* infection, albendazole has parasitologic and clinical efficacy and reduces the risk of relapse.

*Encephalitozoon intestinalis* (formerly *Septata intestinalis*) is a microsporidal species that has recently been recognized as an opportunistic pathogen in patients with AIDS [1, 2]. *E. intestinalis* is a spore-forming protozoan parasite that predominantly infects the epithelial cells of the intestine and the biliary tract. As a result of infection of tissue macrophages, this parasite may also disseminate to other tissues, such as the urinary tract [3]. The main clinical manifestations of *E. intestinalis* infections are chronic diarrhea and cachexia that can be associated with cholangitis, sinusitis, bronchitis, conjunctivitis, and interstitial nephritis [3–5]. Diagnosis of intestinal microsporidiosis can now be reliably made by detection of spores in stool samples using appropriate staining techniques [6, 7]. *E. intestinalis* infection is suspected when large spores are also found in the urine sediment, a unique feature of the genus *Encephalitozoon*. Definitive species identification requires electron microscopy or, more recently, polymerase chain reaction (PCR) analysis of intestinal biopsies, stools, or urine samples [1, 8–10].

Uncontrolled studies have suggested that patients with *E. intestinalis* infections can experience symptomatic improvements with albendazole associated with clearance of the parasite from stools and follow-up intestinal biopsies [3–5, 7, 11–14]. Symptomatic illness may, however, recur following completion of therapy [4, 5, 7, 12].

The present report describes the results of a randomized study of albendazole for the treatment and prophylaxis of *E. intestinalis* infections. Given the results of the first interim analysis, the independent study committee recommended that enrollment of additional patients be discontinued.

Materials and Methods

**Participants.** To be enrolled in the study, human immunodeficiency virus antibody–positive patients had to be ≥18 years old, have microsporidal spores detected on two consecutive stool and urine examinations, have diarrhea (defined as three or more loose or liquid stools per day for at least 3 days), and/or use antidiarrheal medications.

Patients with any of the following findings were excluded: pregnancy or breast-feeding, absolute neutrophil count of <750 × 10⁶/L, platelet count of <50 × 10⁹/L, hemoglobin level of <8.0 g/dL, serum creatinine concentration of >170 μmol/L, serum amino-transferase and alkaline phosphatase levels >5 times the upper limit of normal, and known hypersensitivity to albendazole. Lack of microsporidial infection in intestinal biopsies, identification of a microsporidal species other than *E. intestinalis*, treatment with albendazole or other benzimidazole compounds in the month before enrollment, and antiretroviral therapy started <2 months before enrollment were also criteria for exclusion.

**Design.** The first part of the study was a double-blind placebo-controlled randomized trial intended to assess the efficacy and safety of albendazole as treatment for *E. intestinalis* infection.
Patients received either albendazole (400 mg twice daily with meals) or placebo orally for 3 weeks. Albendazole and placebo were provided by SmithKline Beecham Laboratories (Nanterre, France). At the end of this double-blind period, patients with persistent diarrhea and stool samples positive for microsporidial spores received open-labeled albendazole (400 mg twice daily) for a further 3 weeks.

Patients who cleared microsporidia from stools and follow-up duodenal biopsies were eligible for the second part of the study, which was intended to study the efficacy and safety of maintenance therapy with albendazole as prophylaxis for *E. intestinalis* infection. Patients were randomized to receive either open-labeled albendazole (400 mg twice daily) or no maintenance therapy for a 12-month period or until parasitologic relapse or death.

Use of nonspecific anti diarrheal medications during the study was permitted and recorded in the patient’s stool diary. Patients were allowed to self-adjust their anti diarrheal treatment.

**Diagnosis of *E. intestinalis* infection.** Microsporidial spores were detected in stool and urine samples by using two different staining methods [6, 7]. A semiquantitative assessment of the number of spores per stool smear was performed as previously described [15]. All eligible patients underwent upper gastrointestinal endoscopy with duodenal biopsy to confirm the infection and to identify the species involved using electron microscopy as previously described [8, 15, 16]. Species identification was also confirmed by PCR with primer sets specific for *E. intestinalis*, as previously reported [9, 10].

**Management and follow-up of *E. intestinalis* infection.** Baseline investigations included complete history and physical examination, complete blood cell count, CD4 T lymphocyte count, urinalysis, and serum biochemistry analyses (electrolytes and liver function tests). All participants underwent routine stool studies before enrollment, including bacterial stool cultures, examination for ova and parasites (including cryptosporidia), and toxin assays for *Clostridium difficile*. Patients were requested to keep a stool diary during the study period, as previously reported [15].

Patient assessments were done 2 weeks before initiation of the allocated drug regimen, the day the study regimen was prescribed, and at 2 and 3 weeks after the initiation of the study regimen. At each visit, clinical (physical examination, weight, and stool diary record) and biologic (complete blood cell count, serum biochemistry analyses, and stool and urine examinations) parameters were recorded.

In case of parasitic clearance from follow-up stool specimens after either the double-blind period or open-labeled albendazole, a second gastroduodenal endoscopy with duodenal biopsy was performed to confirm parasitic eradication. Biopsies were handled as for baseline assessment. Once parasitic eradication was confirmed, the patient was eligible for the prophylaxis study.

**Prophylaxis of *E. intestinalis* infection.** Patients were assessed (see above) monthly following randomization in this open-labeled study. In case of parasitic relapse as determined from stool samples, a second sample was obtained shortly thereafter to confirm the relapse.

**Efficacy end points for treatment.** The primary end point was parasitic eradication from the intestinal tract, defined as two consecutive stool samples negative for microsporidial spores and lack of microsporidia in all follow-up duodenal biopsies. Secondary end points included semiquantitative assessment of the number of spores in stools, parasitic clearance from the urinary tract, body weight change, fever, stool frequency, stool consistency, and use of antidiarrheal medications.

**Efficacy end points for prophylaxis.** The primary end point was the time to parasitic relapse from the intestinal tract, defined as two consecutive stool samples positive for microsporidial spores during follow-up. As this prophylaxis study was not blinded, all assessments were performed by physicians blinded to the treatment.

**Safety end points.** All adverse events were recorded, regardless of their relationship to study drugs. Toxicity was graded according to the World Health Organization scoring system. Safety was assessed by incidence, type, and severity of adverse events.

**Statistical analyses.** Comparison of efficacy between treatment groups was based on the percentages of patients who cleared their infection compared by a one-sided Fisher’s exact test. This analysis was based on a modified intention-to-treat approach that included all eligible subjects. The planned sample size was 10 patients per group. Nonparametric paired Kruskal-Wallis tests for continuous variables and Fisher’s exact tests for binary variables were used for comparing secondary end points. P < .05 defined statistical significance. An interim analysis was planned after 10 randomized patients completed the double-blind study.

For statistical analysis of prophylaxis measures in patients with parasitic eradication, the Kaplan-Meier method estimated the time-to-relapse from the monthly parasitic stool examinations. The 2 groups were compared by a one-sided log-rank test. Statistical analysis used SAS software (SAS Institute, Carey, NC).

**Results.**

**Patients.** From September 1994 to November 1996, 10 patients were enrolled in the study. Electron microscopy analysis of duodenal biopsies and PCR results from stools and intestinal biopsies showed that 2 patients were not infected with *E. intestinalis* but with *Enterocytozoon bieneusi* and *Encephalitozoon hellem* (data not shown). These 2 patients were excluded from the analysis.

*E. intestinalis* infection was confirmed in 8 patients. Histopathologic and parasitologic evaluations and electron microscopic examination of duodenal biopsies from 7 patients yielded microsporidia. Electron microscopic examination detected microsporidia with typical ultrastructural features of the developmental stages of *E. intestinalis* in 7 of 8 cases [1]. In 1 case, only duodenal biopsy submitted for parasitologic examination yielded microsporidia. Electron microscopic and histopathologic analyses failed to detect microsporidia but did show changes suggestive of *E. intestinalis* infection of the duodenal mucosa, such as infiltration of the lamina propria with numerous macrophages. Furthermore, *E. intestinalis* DNA could be amplified from this biopsy specimen by PCR, as well as from stool, urine, and bile samples from the same patient (data not shown).

Microsporidial spores could be found in urine (7/8 patients) and also sputum, bronchoalveolar lavage fluid, nasal passages, conjunctiva, and bile duct (1 patient each). PCR assays con-
firmed electron microscopic species identification in 6 of the 7 other patients, and no amplification was obtained from the last patient [9, 10]. No coinfection with another intestinal pathogen was found in 7 patients before or during therapy. Only 1 patient had concomitant Cytomegalovirus duodenitis and C. difficile in stools.

All patients were homosexual men, all but 1 had already experienced AIDS-defining opportunistic diseases, and all had <55 CD4 cells/mm³. All patients had a history of chronic intermittent diarrhea (mean duration, 1 month; range, 10–90 days). At the time of randomization, all patients had diarrhea and abdominal discomfort. Fever (>38°C) was documented in 4 patients, cholangitis in 2, crampy abdominal pain in 1, nausea and vomiting in 2, and bronchitis and sinusitis in 2. Significant leukocyturia (20–200/high-power field) was found in 4 patients associated with increase in creatinine levels in 2. Six patients had increased levels of alkaline phosphatase, and 4 had elevated levels of liver aminotransferases.

Treatment of E. intestinalis microsporidiosis. There were no imbalances between study groups in baseline characteristics (table 1). Mean duration of treatment in the placebo group was 13.5 days (range, 4–21) versus 21 days in the albendazole group. Lack of efficacy with persistent diarrhea was the reason for premature discontinuation in the placebo group. Parasitologic assessments were, however, available for these patients. Microsporidial spores were always detected in follow-up stool and urine specimens from patients randomized to receive placebo with no change in the semiquantitative number of spores (data not shown). By contrast, clearance of microsporidial spores was observed in follow-up stool specimens from the 4 patients randomized to receive albendazole. Urine specimens from 2 of these 4 patients still yielded microsporidia, although the spores appeared to be altered. These 4 patients underwent a second duodenal endoscopy with biopsies. These follow-up biopsies failed to yield microsporidia when examined by histopathology and electron microscopy in 3 of 4 patients. In the 4th patient, electron microscopy only revealed what seemed to be remnant altered microsporidal spores. Eradication of intestinal E. intestinalis infection was therefore obtained in these 4 patients (100% vs. 0%, P = .01, one-sided Fisher’s test).

Parasitologic clearance was associated with clinical efficacy. All patients randomized to receive albendazole gained weight during the 3-week study period (mean, 4.1 kg; range, 2–7.5 kg). By contrast, weight remained stable or decreased in the placebo group (mean, −1.4; range, 0 to −4 kg). These differences were significant (P = .01, one-sided Kruskal-Wallis test). Furthermore, reductions in stool frequency and in use of antimotility drugs were noted more frequently in the albendazole group than the placebo group, but these changes were not significant. Two patients in the albendazole group were receiving antimotility drugs at the beginning of the trial; by the end of treatment, 1 had stopped this medication, and the other had reduced the dose. Only 1 patient in the placebo group had used antimotility drugs at baseline, but two did at the end of treatment. Similarly, all patients in the albendazole group had formed stools at the end of the treatment compared with none in the placebo group (100% vs. 0%, P = .01, one-sided Fisher’s exact test). Fever resolved in 1 (1/1) patient in the albendazole group but persisted in 3 of 3 patients in the placebo group. Also, decreases in alkaline phosphatase and creatinine levels were noted in the albendazole group (2/3 and 2/2, respectively) and associated with the disappearance of leukocyturia in 3 of 3 patients.

Open-labeled albendazole was then offered to the 4 patients in the placebo group who did not clear microsporidia from stools. All completed a 21-day course of albendazole and cleared E. intestinalis from follow-up stools and intestinal biopsies. The overall cure rate of the albendazole regimen in our study was therefore 100% (8/8; 95% confidence interval, 0.64–1). Urine analysis still yielded microsporidial spores in 3 of these patients at the end of the treatment.

Prophylaxis. After albendazole therapy, all 8 patients had cleared microsporidial infection from their intestinal tract and were randomly assigned to receive either maintenance therapy with albendazole (400 mg twice daily: 3 patients) or no treatment (5 patients). Two patients in the albendazole group and 3 in the no-treatment group still had microsporidia in urine samples at that time.

The 2 groups remained well-balanced with respect to age, sex, AIDS-defining diseases, CD4 cell count, and duration of previous albendazole therapy (each patient had received 3 weeks of albendazole). Four patients received protease inhibitors (ritonavir or indinavir) during this trial, 2 in each group, starting a mean of 32 days (range, 0–82) after randomization. All 4 patients had similar CD4 cell counts.

During the study period, none of the 3 patients receiving maintenance therapy had a recurrence of E. intestinalis infection (mean follow-up, 7.7 months; range, 6–9). Microsporidial spores were no longer detected in the urine samples from these patients. Three relapses were recorded among the 5 patients receiving no prophylaxis, including both receiving protease inhibitors (mean follow-up, 2.7 months; range, 2–3.3). The 2
other patients died without relapse after a 4-month follow-up. The comparison of the failure times between the 2 groups was significant ($P = .04$, one-sided log-rank test).

Two of these relapses occurred in patients with persistent asymptomatic shedding of microsporidial spores in urine. Parasitologic relapse was associated with diarrhea in only 1 patient who had concomitant cryptosporidiosis. All 3 patients who relapsed received a further 3-week course of albendazole therapy with parasitologic clearance.

Death occurred in 4 patients during follow-up (in 3 receiving no prophylaxis and in 1 receiving albendazole). By log-rank analysis, albendazole had no significant effect on mortality during follow-up. Deaths were due to AIDS-related diseases in 3 patients (non-Hodgkin’s lymphoma, cerebral tumor, human immunodeficiency virus encephalopathy) and to staphylococcal bacteremia in 1.

Adverse events (table 2). Only one serious adverse event was reported during the double-blind period of the trial and involved a patient with concomitant Cytomegalovirus duodenitis, randomized to the placebo group, who developed bacterial pneumonia and seizures after 17 days of treatment. This patient recovered after receiving broad-spectrum antibiotic therapy.

Ten nonserious adverse events were also reported during the double-blind study, mostly headaches and gastrointestinal adverse events (table 2). No long-term safety problems were recorded during maintenance therapy with albendazole.

Discussion

Albendazole is a broad-spectrum antiparasitic agent with activity against protozoa, cestodes, and nematodes [17]. Albendazole binds to the colchicine-sensitive site of tubulin, inhibiting its polymerization into microtubules, thus interfering with nutrient uptake and cell division [18]. Albendazole has been previously used in patients with AIDS for the treatment of infection with E. bieneusi, another microsporidial species [19, 20]. Although symptomatic improvement was noted in some patients, there was no reduction in parasite burden, as spores were always detected in stools and follow-up duodenal biopsies. In contrast, E. intestinalis appears to be particularly sensitive to albendazole. Previous reports have suggested the efficacy of albendazole in patients with E. intestinalis infections, although one parasitologic and two clinical failures were recorded among 22 reported cases [3, 5, 7, 11–14]. Also, no albendazole regimen can be clearly recommended from these studies, as different doses and treatment durations were used.

Our double-blind placebo-controlled trial has demonstrated, with only 8 randomized patients, that albendazole is an effective treatment for E. intestinalis microsporidiosis in patients with AIDS. Clearance of E. intestinalis, as assessed by repeated stool examination and follow-up duodenal biopsies, was achieved in all patients receiving albendazole compared with none of the patients receiving placebo. Parasitologic clearance was also associated with clinical benefit. The efficacy of albendazole is further supported by the fact that patients initially randomized to receive placebo cleared their microsporidial infections following open-labeled albendazole. Overall, as 8 of 8 patients cleared their infection in our study, the cure rate with albendazole was 100% (95% confidence interval, 0.64–1).

A randomized double-blind controlled trial of albendazole (800 mg twice daily for 2 weeks) in patients with AIDS and chronic diarrhea has also been conducted in Zambia and has shown remission of diarrhea in 26% of the patients receiving albendazole [21]. Albendazole was used empirically, however, without any prior stool investigation. Since albendazole has broad activity against Strongyloides stercoralis, Giardia intestinalis, and microsporidia, all of which can cause diarrhea in patients with AIDS, no definitive conclusion can be drawn from this trial about the efficacy of albendazole specifically on microsporidial infection [21]. Adverse drug reactions were mild during this study and never required the discontinuation of therapy.

The only serious adverse event during our study was observed in a patient randomized to receive placebo. Only mild increases in aminotransferases could be associated with albendazole therapy. The dosage of albendazole used in this study was similar to that used in hydatid cyst disease, in which up to 12 cycles of 28-day treatments have been given. Only rare side effects were noted in that study, including nausea, alopecia, rash, liver function abnormalities, and neutropenia [22].

Our second objective was to assess the efficacy of long-term maintenance therapy with albendazole to prevent relapses of this microsporidial infection. Indeed, although the efficacy of albendazole in the treatment of E. intestinalis infections had been suggested by previous studies, a number of relapses were also noted within months of completing therapy, some of which occurred in patients still receiving low-dose albendazole [4, 7, 11].

We therefore designed a randomized study to compare albendazole (400 mg twice daily) with no treatment to investigate the ability of albendazole to prevent recurrent microsporidiosis and demonstrated that albendazole significantly delayed the occurrence of relapse.
The persistent shedding of spores in urine might be associated with relapse because two of the three relapses occurred among the 3 patients who had microsporidial spores in urine detected at randomization and who received no treatment. We have yet no explanation for the persistent shedding of spores in urine in patients receiving albendazole since the active sulfone metabolite of albendazole is eliminated in the urine. Further studies are needed to clarify this issue.

In the light of the morbidity due to recurrent diarrhea and the good safety profile of albendazole, we believe that continuing prophylaxis should be indicated after an initial treatment of *E. intestinalis* infection. Another alternative would be to closely monitor stool samples and to propose a new course of albendazole once microsporidial spores are again detected. Indeed, a new course of albendazole eradicated the infection in the 3 patients who relapsed during the study.

Our results might be extended to other *Encephalitozoon* species (*E. cuniculi, E. hellem*) infecting patients with AIDS who are sensitive to albendazole in vitro [23]. There are indeed preliminary data suggesting that patients with disseminated *Encephalitozoon* infection improved clinically and stopped excreting parasites when treated with albendazole [24].

We conclude that *E. intestinalis* infections in patients with AIDS can be treated effectively with a 3-week course of albendazole and that recurrent disease can subsequently be prevented by long-term albendazole maintenance therapy.

Acknowledgments

We thank Jean Dormont and Jean-Marie Decazes for helpful discussions, Marc Lemann for performing duodenal endoscopies, Agnès Lesourd and Francois Rocher for the preparation and analysis of the biopsy specimens, Delphine Bourin from the pharmacy, Sabine Bréchignac, Stéphane Roman, Bernard Doumenc, and Michel Janier for the referral of patients, and H. Boudjadia from SmithKline Beecham Pharmaceuticals for providing albendazole and placebo.

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