

FUMAGILLIN TREATMENT OF INTESTINAL MICROSPORIDIOSIS

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ABSTRACT

Background Intestinal microsporidiosis due to *Enterocytozoon bieneusi* is a cause of chronic diarrhea, malabsorption, and wasting in immunocompromised patients. Currently, there is no effective treatment.

Methods We conducted a randomized, double-blind, placebo-controlled trial of fumagillin (60 mg per day orally for two weeks) in patients with chronic *E. bieneusi* infection. Efficacy was assessed primarily by the clearance of microsporidia, as evidenced by analysis of stool specimens. Patients in whom microsporidia were not cleared received treatment for two weeks with open-label fumagillin. After clearance of the parasite, follow-up stool examinations were performed monthly to detect relapses.

Results Twelve patients were enrolled in this study, 10 with the acquired immunodeficiency syndrome and 2 who had received organ transplants. Clearance of microsporidia occurred in all six of the patients in the fumagillin group, as compared with none of the six in the placebo group ($P=0.002$). Treatment with fumagillin was also associated with increases in absorption of D-xylose ($P=0.003$) and in Karnofsky performance scores ($P<0.001$) and with decreases in loperamide use ($P=0.01$) and total stool weight ($P=0.04$). There were serious adverse events (neutropenia and thrombocytopenia) in three patients in the fumagillin group; one patient in the placebo group had severe diarrhea. All six controls subsequently had clearance of microsporidia after open-label treatment with fumagillin. Relapses of the infection were identified in two patients during follow-up (median follow-up, 10 months).

Conclusions Fumagillin is an effective treatment for chronic *E. bieneusi* infection in immunocompromised patients. (N Engl J Med 2002;346:1963-9.)

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ENTEROCTOZOON BIENEUSI, a spore-forming, eukaryotic, unicellular parasite, has been recognized as an opportunistic enteric pathogen in patients with the acquired immunodeficiency syndrome (AIDS).¹⁻⁵ Intestinal microsporidiosis can cause chronic diarrhea, malabsorption, and weight loss in immunocompromised patients.²⁻⁸ The diagnosis of intestinal microsporidiosis can now be made reliably on the basis of the detection of spores in stool samples with the use of appropriate staining.^{9,10} Definitive identification of the species,

however, requires amplification of DNA by the polymerase chain reaction (PCR).¹¹ Several agents have been tested as treatments for this infection, but they do not lead to clearance of *E. bieneusi*, as evidenced by analysis of stool specimens.^{12,13} Immune reconstitution has been associated with remissions of *E. bieneusi* infections in patients with human immunodeficiency virus (HIV) infection who are receiving highly active antiretroviral therapy.^{14,15}

Fumagillin, an antibiotic derived from the fungus *Aspergillus fumigatus*, has been used to treat microsporidiosis in honeybees,¹⁶ and it has been effective in vitro against microsporidia.¹⁷⁻²⁰ In humans, fumagillin was used more than 40 years ago for the treatment of intestinal amebiasis,^{21,22} and it is effective when used topically in the treatment of microsporidial keratoconjunctivitis.²³⁻²⁵

In preliminary studies, oral fumagillin has had efficacy in HIV-infected patients with *E. bieneusi* infection.^{26,27} We undertook this study in order to assess the efficacy of fumagillin in the treatment of intestinal microsporidiosis in immunocompromised patients.

METHODS

Study Population

The study was approved by the ethics committee of the Saint-Louis Hospital in Paris. Enrollment began in June 1999, and written informed consent was obtained from all patients.

All participants were 18 years old or older and were immunocompromised because of infection with HIV type 1 (HIV-1) or the transplantation of bone marrow or an organ. Patients had chronic intestinal microsporidiosis, defined by the presence of microsporidia on two consecutive stool examinations two weeks before randomization. Patients were excluded if they had begun receiving a new antiretroviral agent or if their immunosuppressive regimen had been changed less than two months before enrollment. Other criteria for exclusion were concomitant treatment with antiarrhythmic agents or drugs that might increase the risk of thrombocytopenia or bleeding (e.g., aspirin or chemotherapeutic regimens), might affect the metabolism or absorption of fumagillin (e.g., rifampin, rifabutin, phenobarbital, cimetidine, phenytoin, or antacids), or might have activity against microsporidia (e.g., azole compounds). Patients were

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also excluded if they had any of the following laboratory abnormalities: a platelet count of less than 150,000 per cubic millimeter, an absolute neutrophil count of less than 1000 per cubic millimeter, a hemoglobin level below 8.0 g per deciliter, a serum creatinine concentration of less than 170 μmol per liter, serum levels of aminotransferases and alkaline phosphatase more than three times the upper limit of normal, or a serum lipase level more than two times the upper limit of normal.

Diagnosis of *E. bienersi* Infection

All stool samples were analyzed in a central laboratory. *E. bienersi* infection was suggested by the identification of typical spores in stools with the use of two different staining methods, and the identification of the species was confirmed by PCR, as previously reported.⁹⁻¹¹ For a semiquantitative assessment of the number of spores per stool sample, the scoring system was as follows: 0 indicated no spores; 1 indicated rare spores; 2 indicated some spores; and 3 indicated numerous spores.²⁷

Study Design and Treatment

The first phase of the study was a double-blind, placebo-controlled, randomized trial. Patients were randomly assigned in a 1:1 ratio to receive either fumagillin (20 mg three times daily on an empty stomach) or placebo orally for two weeks. Fumagillin and placebo were provided by Sanofi-Synthelabo Laboratories. After this double-blind phase (two weeks after the end of treatment [week 4]), patients with microsporidial spores in their stools received open-label fumagillin (20 mg three times a day) for an additional two weeks.

Patients whose stools no longer contained spores were then followed with monthly stool examinations, until parasitologic relapse or death, for a maximum of 12 months. Concomitant medications, including prophylaxis against opportunistic infections, were allowed. Antiretroviral therapy and immunosuppressive regimens were also allowed, provided that they were not modified during the placebo-controlled phase of the trial. Patients were also allowed to use anti-diarrheal medications and to adjust their own doses of such medications.

Treatment and Follow-up

Base-line investigations included a complete medical history and physical examination, a complete blood count, urinalysis, biochemical analysis of serum, bacterial stool cultures, examination of stool for ova and parasites (including cryptosporidia), and assays for the *Clostridium difficile* toxin.

Patients were also assessed one, two, and four weeks after the initiation of fumagillin or placebo. Clinical and biologic measurements were recorded at each visit. Patients were asked to use a diary to record the number of bowel movements, the characteristics of their stools, the use of anti-diarrheal medications, and any adverse events. The number and characteristics of bowel movements and the total weight of stools during two consecutive days before the visits on day 1, at week 2, and at week 4 were recorded. In addition, complete blood counts were obtained on days 10, 12, 17, and 19. The results of platelet counts, however, were not given to the investigators until week 4, unless the count decreased below 75,000 per cubic millimeter, resulting in the interruption of the study treatment. This procedure was used in order to keep the investigators unaware of the treatment-group assignment, since fumagillin had induced frequent thrombocytopenia in previous trials.^{26,27} At base line and at week 4, the blood D-xylose concentration was measured in a central laboratory 90 minutes after the oral administration of 25 g of D-xylose in order to assess intestinal absorption.⁴ Examinations of stool specimens for microsporidia were performed on days 1, 8, 15, 17, and 19. Similar follow-up was performed during the open-label phase of the trial, except that the investigators were aware of the results of stool examinations and platelet counts.

Efficacy End Points

Clearance of parasites, which was the primary efficacy end point, was defined as the absence of microsporidial spores in stools 15 and 17 days after either double-blind or open-label treatment. If there were conflicting results, a third stool sample was obtained on day 19 and analyzed. Laboratory staff members who analyzed the stool samples were unaware of the treatment-group assignment.

Secondary end points included semiquantitative assessments of the number of spores in stools and changes in body weight, stool weight, stool frequency and consistency, blood D-xylose levels, and use of anti-diarrheal medications during the study period. All assessments were performed by physicians who were unaware of the treatment-group assignment, the results of stool examinations, and the platelet counts until week 4 of the double-blind phase. Parasitologic relapse was defined by the reemergence of spores in follow-up stool samples.

Safety End Points

All adverse events were recorded, regardless of their relation to the study drugs. Toxic effects were graded according to the scoring system of the World Health Organization (WHO). A serious adverse event was defined as any event not related to HIV infection or organ transplantation that was fatal or life-threatening, resulted in hospital admission or prolonged hospitalization, or caused permanent disability, cancer, or congenital anomaly; any clinical or biologic event of WHO grade 3 or 4; or overdose of the study drug.

Statistical Analysis

All analyses were performed according to the intention-to-treat principle. Analysis of the primary efficacy end point involved the comparison by Fisher's exact test of the percentages of patients in the two treatment groups in whom clearance of the parasites occurred. In the analysis of secondary end points, Wilcoxon signed-rank tests for paired nonparametric data were used for numerical variables and Fisher's exact tests were used for categorical variables. Analysis of variance and logistic-regression models were also used to assess the effect of randomization on secondary end points, while incorporating the effects of time. We used Kaplan-Meier analysis to estimate the time to relapse on the basis of the results of the monthly stool examinations.

We determined that we needed a sample of 10 patients per group, assuming an estimated rate of clearance of parasites of 80 percent with fumagillin and 10 percent with placebo, with alpha and beta levels of 5 percent and two-tailed tests. We planned to conduct the first interim analysis of efficacy and safety after 10 patients had completed the trial. On October 16, 2000, the data and safety monitoring board reviewed the data and recommended that the enrollment of patients be discontinued. The decision was endorsed by the scientific committee for the trial.

All P values reported are two-sided. A P value of less than 0.02 was considered to indicate statistical significance for the primary end points, because of the one interim analysis that had been planned and conducted before stopping the study. Data are reported as medians and interquartile ranges, unless otherwise indicated. Statistical analysis was performed with the use of SAS software, version 8 (SAS Institute). The study was designed and completed by the investigators of the Agence Nationale de Recherches sur le SIDA 090 study, who held the data and performed all statistical analyses. The sole role of Sanofi-Synthelabo was to provide active drug and placebo for the trial.

RESULTS

Study Patients

Between June 1999 and November 2000, 19 patients were screened and 12 underwent randomiza-

tion. Reasons for not undergoing randomization included death before randomization (in the case of one patient), thrombocytopenia (three patients), alterations in the results of liver-function tests (two patients), and absence of microsporidia in stools (one patient).

The base-line characteristics of the 12 patients who underwent randomization are summarized in Table 1. Of these patients, 10 had HIV infection and had a median CD4 count of 16 per cubic millimeter (range, 4 to 99 per cubic millimeter). Three of these patients had not received antiretroviral medication. All were homosexual men with AIDS and had had multiple opportunistic diseases. The two remaining patients had undergone organ transplantation; one had received a kidney transplant 39 months before enrollment and was receiving 2 mg of tacrolimus and 10 mg of prednisone per day, and the other had received a liver transplant 21 months before enrollment and was receiving 2 mg of tacrolimus per day.

In all 12 patients, typical microsporidial spores were detected before the initiation of treatment, and *E. bienersi* DNA was amplified from stools. The median duration of microsporidial infection in these patients was 25 weeks.

The treatment groups were well balanced in terms of the base-line characteristics of the patients. At enrollment, all patients reported diarrhea, bloating, and mild abdominal discomfort. All patients had passed at least one liquid stool in the two days before the visit on day 1, and the total weight of stools was more than 300 g per day in all patients (data not shown). Associated intestinal pathogens (entamoeba species) were found in only one patient. D-Xylose-absorption tests were abnormal at base line in all but one patient (who was assigned to the placebo group).

Primary End Point

The patients were followed as required by the study protocol. No patient was lost to follow-up, and none prematurely stopped the study treatment. The shedding of *E. bienersi* spores in stools ceased in all of the six patients in the fumagillin group, as compared with none in the placebo group (100 percent vs. 0 percent, P=0.002 by Fisher's exact test) (Table 2).

Secondary End Points

Microsporidial clearance was associated with a significant increase in D-xylose absorption in the fumagillin group but not in the placebo group (P=0.003), with normal D-xylose-absorption tests in five of the six patients in the fumagillin group at week 4 but in none of the six patients in the placebo group (P<0.02) (Table 2).

Microsporidial clearance in the fumagillin group was also associated with clinical efficacy. Indeed, there

TABLE 1. BASE-LINE CHARACTERISTICS OF THE 12 PATIENTS WITH INTESTINAL MICROSPORIDIOSIS.*

CHARACTERISTIC	PLACEBO GROUP (N=6)	FUMAGILLIN GROUP (N=6)	P VALUE
Male sex — no. (%)	6 (100)	6 (100)	1.00
Age — yr			0.30
Median	42	36	
Interquartile range	37–45	33–45	
Body-mass index†			0.58
Median	18	18	
Interquartile range	17–20	18–20	
Body weight — kg			0.75
Median	56.9	53.3	
Interquartile range	47.5–61.0	50.0–58.0	
Karnofsky score‡			0.25
Median	70	80	
Interquartile range	70–80	80–100	
Risk factors for microsporidial infection			
Organ transplantation — no. (%)	0	2 (33)	0.45
HIV infection — no. (%)	6 (100)	4 (67)	
Had received HAART — no. (%)	5 (83)	2 (50)	0.50
Had not received antiretroviral drugs — no. (%)	1 (17)	2 (50)	
CD4 count — cells/mm ³			1.00
Median	15	28	
Interquartile range	10–48	12–44	
Blood D-xylose concentration — g/liter§			0.81
Median	0.10	0.08	
Interquartile range	0.04–0.14	0.08–0.10	

*HIV denotes human immunodeficiency virus, and HAART highly active antiretroviral therapy.

†The body-mass index is the weight in kilograms divided by the square of the height in meters.

‡The Karnofsky performance score, a measure of functional ability, ranges from 0 to 100, with higher scores indicating better performance.

§The normal value is more than 0.25 g per liter.

was a trend toward lower median total weight of stools in the fumagillin group than in the placebo group (P=0.04). At week 4, no patient in the fumagillin group was using loperamide, whereas four of the six patients in the placebo group still required this antimotility drug (P=0.009). There was also a trend toward more solid stools in the fumagillin group than in the placebo group (P=0.06). However, there were no clear changes noted in the number of bowel movements per day or in body weight. There was a significant increase in Karnofsky scores in the fumagillin group, whereas there was a decrease in the placebo group (P<0.001). New or relapsing opportunistic infections were recorded in three patients during the double-blind phase (pulmonary aspergillosis in one patient in the fumagillin group, disseminated histoplasmosis in

TABLE 2. STUDY END POINTS IN THE 12 PATIENTS WITH INTESTINAL MICROSPORIDIOSIS.

VARIABLE	PLACEBO GROUP (N=6)	FUMAGILLIN GROUP (N=6)	P VALUE	VARIABLE	PLACEBO GROUP (N=6)	FUMAGILLIN GROUP (N=6)	P VALUE
Patients with clearance of parasites by days 15 and 17 — no. (%)	0 (0)	6 (100)	0.002	Loperamide capsules taken in 2 days — no.			0.01
No. of spores in stools*			0.005	Base line	3	4	
Base line				Median			
Median	3	3		Interquartile range	1–12	0–6	
Interquartile range	2–3	2–3		Week 2			
Week 2				Median	4	0	
Median	2	0		Interquartile range	0–12	0–1	
Interquartile range	2–2	0–0		Week 4			
Day 19				Median	2	0	
Median	2	0		Interquartile range	1–12	0–0	
Interquartile range	2–3	0–0		Blood D-xylose concentration — g/liter			0.003
Stool weight — g†			0.04	Base line			
Base line				Median	0.10	0.08	
Median	1450	1225		Interquartile range	0.04–0.14	0.08–0.10	
Interquartile range	670–2540	994–1428		Week 4			
Week 2				Median	0.10	0.30	
Median	1662	653		Interquartile range	0.06–0.14	0.25–0.41	
Interquartile range	645–2095	619–880		Body weight — kg			0.26
Week 4				Base line			
Median	1112	715		Median	57	53	
Interquartile range	327–2750	425–738		Interquartile range	48–61	50–58	
Stool frequency per 2-day period			0.90	Week 2‡			
Base line				Median	59	53	
Median	9	7		Interquartile range	53–65	52–58	
Interquartile range	6–13	5–10		Week 4			
Week 2				Median	56	55	
Median	5	6		Interquartile range	47–66	52–61	
Interquartile range	4–7	4–8		Karnofsky score			<0.001
Week 4				Base line			
Median	5	4		Median	70	80	
Interquartile range	2–10	3–5		Interquartile range	70–80	80–100	
Patients with at least one liquid stool — no. (%)			0.06	Week 2			
Base line	6 (100)	6 (100)		Median	70	95	
Week 2	5 (83)	1 (17)		Interquartile range	40–70	90–100	
Week 4	4 (67)	2 (33)		Week 4			
Patients taking loperamide — no. (%)			0.009	Median	55	100	
Base line	6 (100)	4 (67)		Interquartile range	30–90	90–100	
Week 2	3 (50)	1 (17)					
Week 4	4 (67)	0 (0)					

*The number of spores was assessed semiquantitatively as described in the Methods section.

†Data are totals for two days.

‡The weight at week 2 was missing for one patient.

one patient in the placebo group, and cytomegalovirus disease in one patient in the placebo group).

Open-Label Fumagillin and Follow-up

After the double-blind phase of the study, open-label fumagillin was offered to the six patients in the placebo group, in whom clearance of microsporidia had not occurred. Three patients prematurely discontinued the study treatment (one each 11, 13, and 14 days after open-label treatment began) because of thrombocytopenia. In all patients, however, follow-up

stool specimens did not contain *E. bienersi*. The overall cure rate associated with the fumagillin regimen in our study was therefore 100 percent (95 percent confidence interval, 76 to 100 percent).

The median duration of follow-up after fumagillin therapy was 10 months (range, 5 to 12). One patient died from end-stage HIV disease five months after the end of treatment without having had a relapse. His CD4 count was only 10 cells per cubic millimeter. Two parasitologic and clinical relapses were recorded one month after treatment was completed in the HIV-

infected patients. At day 40 of follow-up, the estimated rate of relapse was 16.7 percent (95 percent confidence interval, 0 to 38.3 percent). The two patients with relapses received a second course of fumagillin through an expanded-access program. Both had clearance of parasites, but one later had another relapse. No relapse was documented during follow-up in the other patients. The last available CD4 counts were only 6, 35, 72, and 95 cells per cubic millimeter in the HIV-infected patients who had received highly active antiretroviral therapy before the trial.

Adverse Events

Five serious adverse events were reported (in four patients) during the double-blind phase of the trial (Table 3). One patient in the placebo group was admitted to the hospital because of severe diarrhea and weight loss. Three patients in the fumagillin group had laboratory abnormalities that did not require hospital admission: one patient had grade 4 neutropenia and grade 4 thrombocytopenia, one had grade 4 neutropenia alone, and one patient had grade 3 thrombocytopenia. Both patients with neutropenia had already had grade 4 neutropenia at base line. Platelet counts, however, were above 150,000 per cubic millimeter in all patients at base line. Thrombocytopenia and neutropenia completely resolved within 8 to 14 days after the cessation of fumagillin and other hematotoxic agents. No bleeding or purpura was noted in patients with thrombocytopenia.

Seventeen grade 2 adverse events were also reported during the double-blind phase of the study — nine in the placebo group and eight in the fumagillin group (Table 3). It was difficult to ascertain, however, whether these events were related to fumagillin therapy or to underlying conditions in these severely immunocompromised patients.

Seven serious adverse events were reported during the open-label phase of the study. One patient with severe diarrhea had grade 3 hypokalemia, and one patient who was receiving foscarnet had diabetes insipidus. In two patients, grade 4 thrombocytopenia developed; one of these patients also had mild epistaxis and anal bleeding, and the other had hemoptysis and received a transfusion of platelets. Grade 4 anemia developed in two patients, both of whom had had low hemoglobin levels at base line. One patient had grade 4 neutropenia.

DISCUSSION

This double-blind, placebo-controlled trial shows that fumagillin is an effective treatment for *E. bienensis* microsporidiosis in immunocompromised patients. Clearance of *E. bienensis*, as assessed by repeated parasitologic examinations of stool samples, was achieved in all patients receiving fumagillin, as compared with

TABLE 3. ADVERSE EVENTS DURING THE DOUBLE-BLIND PHASE OF THE STUDY.*

VARIABLE	PLACEBO GROUP (N=6)	FUMAGILLIN GROUP (N=6)
	no.	
Clinical events		
Grade 3–4		
Diarrhea and weight loss	1	0
Grade 2		
Cough	1	0
Fever	0	1
Abdominal cramps	0	1
Rash, pruritus, or both	1	1
Optic neuritis	1	0
Diffuse edema	1	0
Malaise	0	1
Laboratory abnormalities		
Elevated aminotransferases		
Grade 2 (>2.5 times ULN)	0	1
Elevated alkaline phosphatase		
Grade 2 (>2.5 times ULN)	1	1
Anemia		
Grade 2 (hemoglobin concentration <9.5 g/dl)	3	0
Neutropenia		
Grade 3–4 (<750 neutrophils/mm ³)	0	2
Grade 2 (<1000 neutrophils/mm ³)	1	1
Thrombocytopenia		
Grade 3–4 (<50,000 platelets/mm ³)	0	2
Grade 2 (<75,000 platelets/mm ³)	0	1
Total no. of adverse events	10	12

*ULN denotes the upper limit of normal.

none of the patients receiving placebo. Parasitologic clearance was also associated with normalization of the results of D-xylose–absorption tests and with clinical benefit, as indicated by decreased weight of stools, decreased use of loperamide, and an increase in Karnofsky scores. The efficacy of fumagillin against this infection is further supported by the fact that patients who were initially assigned to placebo had clearance of microsporidial infection after receiving open-label fumagillin. All 12 patients in our study had clearance of infection, resulting in a cure rate with fumagillin of 100 percent (95 percent confidence interval, 76 to 100 percent).

During a median follow-up of 10 months after the completion of fumagillin treatment, there were only two relapses, both in patients with AIDS. Although the three patients who had not received antiretroviral drugs at base line but received highly active antiretroviral therapy during follow-up had immune reconstitution that prevented assessment of the long-term efficacy of fumagillin, there were no relapses in the two transplant recipients or in the four HIV-infected patients whose CD4 counts remained low.

Toxic effects on bone marrow were the principal adverse effect of fumagillin therapy.^{26,27} Thrombocytopenia was the most frequent adverse event, occurring in more patients than any other adverse event, with grade 3 and 4 adverse events reported in 4 of the 12 patients (33 percent). Thrombocytopenia began after one week of treatment, with a nadir three days after the end of treatment. Spontaneous recovery of a normal platelet count was observed in all patients one to two weeks after the discontinuation of fumagillin therapy. The mechanisms of fumagillin-induced bone marrow toxicity might be related to a direct toxic effect of fumagillin on megakaryocytic and myeloid progenitors in vitro.²⁷ Bone marrow toxicity had not been reported in patients with intestinal amebiasis^{21,22} but was reported in patients with cancer and Kaposi's sarcoma, in whom fumagillin was tested as an antiangiogenic agent.^{28,29} Close monitoring of blood counts is therefore mandatory during fumagillin therapy and should be performed every other day from day 8 to day 19, and treatment should be stopped if the platelet count falls below 75,000 per cubic millimeter.

The results of this trial confirm our previous observations^{26,27} and support the use of oral fumagillin as a treatment for *E. bienersi* microsporidiosis in patients with AIDS and other types of immunodeficiency. The mechanisms by which fumagillin inhibits microsporidial replication are, however, poorly understood. Fumagillin may act through the inhibition of methionine aminopeptidase 2 by irreversibly blocking the active site.³⁰⁻³² Further studies are needed to elucidate the mechanism of action of fumagillin and to develop compounds with lower bone marrow toxicity.

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APPENDIX

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REFERENCES

- Desportes I, Le Charpentier Y, Galian A, et al. Occurrence of a new microsporidian: *Enterocytozoon bienersi* n.g., n. sp., in the enterocytes of a human patient with AIDS. *J Protozool* 1985;32:250-4.
- Kotler DP, Orenstein JM. Clinical syndromes associated with microsporidiosis. In: Wittner M, Weiss LM, eds. *The microsporidia and microsporidiosis*. Washington, D.C.: ASM Press, 1999:258-92.
- Eeftinck Schattenkerk JKM, van Gool T, van Ketel RJ, et al. Clinical significance of small-intestinal microsporidiosis in HIV-1-infected individuals. *Lancet* 1991;337:895-8.
- Molina JM, Sarfati C, Beauvais B, et al. Intestinal microsporidiosis in human immunodeficiency virus-infected patients with chronic unexplained diarrhea: prevalence and clinical and biologic features. *J Infect Dis* 1993; 167:217-21.
- Orenstein JM, Chiang J, Steinberg W, Smith PD, Rotterdam H, Kotler DP. Intestinal microsporidiosis as a cause of diarrhea in human immunodeficiency virus-infected patients: a report of 20 cases. *Hum Pathol* 1990; 21:475-81.
- Sax PE, Rich JD, Pieciak WS, Trnka YM. Intestinal microsporidiosis occurring in a liver transplant recipient. *Transplantation* 1995;60:617-8.
- Guerard A, Rabodonirina M, Cotte L, et al. Intestinal microsporidiosis occurring in two renal transplant recipients treated with mycophenolate mofetil. *Transplantation* 1999;68:699-707.
- Gainzarain JC, Canut A, Lozano M, et al. Detection of *Enterocytozoon bienersi* in two human immunodeficiency virus-negative patients with chronic diarrhea by polymerase chain reaction in duodenal biopsy and review. *Clin Infect Dis* 1998;27:394-8.
- Weber R, Bryan RT, Owen RL, et al. Improved light-microscopical detection of microsporidia spores in stools and duodenal aspirates. *N Engl J Med* 1992;326:161-6.
- van Gool T, Snijders F, Reiss P, et al. Diagnosis of intestinal and disseminated microsporidial infections in patients with HIV by a new rapid fluorescence technique. *J Clin Pathol* 1993;46:694-9.
- Liguory O, David F, Sarfati C, et al. Diagnosis of infections caused by *Enterocytozoon bienersi* and *Encephalitozoon intestinalis* using polymerase chain reaction in stool specimens. *AIDS* 1997;11:723-6.
- Dieterich DT, Lew EA, Kotler DP, Poles MA, Orenstein JM. Treatment with albendazole for intestinal disease due to *Enterocytozoon bienersi* in patients with AIDS. *J Infect Dis* 1994;169:178-83.
- Anwar-Bruni DM, Hogan SE, Schwartz DA, Wilcox CM, Bryan RT, Lennox JL. Atovaquone is effective treatment for the symptoms of gastrointestinal microsporidiosis in HIV-1-infected patients. *AIDS* 1996;10:619-23.
- Goguel J, Katlama C, Sarfati C, Maslo C, Lepout C, Molina JM. Remission of AIDS-associated intestinal microsporidiosis with highly active antiretroviral therapy. *AIDS* 1997;11:1658-9.
- Carr A, Marriott D, Field A, Vasak E, Cooper DA. Treatment of HIV-1-associated microsporidiosis and cryptosporidiosis with combination antiretroviral therapy. *Lancet* 1998;351:256-61.
- Katznelson H, Jamieson CA. Control of Nosema disease of honeybees with fumagillin. *Science* 1952;115:70-1.
- Shaddock JA. Effect of fumagillin on in vitro multiplication of *Encephalitozoon cuniculi*. *J Protozool* 1980;27:202-8.
- Beauvais B, Sarfati C, Challier S, Derouin F. In vitro model to assess the effect of antimicrobial agents on *Encephalitozoon cuniculi*. *Antimicrob Agents Chemother* 1994;38:2440-8.
- Didier ES. Effects of albendazole, fumagillin, and TNP-470 on microsporidial replication in vitro. *Antimicrob Agents Chemother* 1997;41: 1541-6.
- Coyle C, Kent M, Tanowitz HB, Wittner M, Weiss LM. TNP-470 is an effective antimicrosporidial agent. *J Infect Dis* 1998;177:515-8.
- McCowen MC, Callender ME, Lawlis JF Jr. Fumagillin (H-3), a new antibiotic with amebicidal properties. *Science* 1951;113:202-3.
- Killough JH, Magill GB, Smith RC. The treatment of amebiasis with fumagillin. *Science* 1952;115:71-2.
- Yee RW, Tio FO, Martinez JA, Held KS, Shaddock JA, Didier ES. Resolution of microsporidial epithelial keratopathy in a patient with AIDS. *Ophthalmology* 1991;98:196-201.
- Diesenhouse MC, Wilson LA, Corrent GF, Visvesvara GS, Grossniklaus HE, Bryan RT. Treatment of microsporidial keratoconjunctivitis with topical fumagillin. *Am J Ophthalmol* 1993;115:293-8.
- Wilkins JH, Joshi N, Margolis TP, Cevallos V, Dawson DR. Microspo-

ridial keratoconjunctivitis treated successfully with a short course of fumagillin. *Eye* 1994;8:703-4.

26. Molina JM, Goguel J, Sarfati C, et al. Potential efficacy of fumagillin in intestinal microsporidiosis due to *Enterocytozoon bieneusi* in patients with HIV-infection: results of a drug screening study. *AIDS* 1997;11:1603-10.
27. Molina JM, Goguel J, Sarfati C, et al. Trial of oral fumagillin for the treatment of intestinal microsporidiosis in patients with HIV infection. *AIDS* 2000;14:1341-8.
28. DiPaolo JA, Tarbell DS, Moore GE. Studies on the carcinolytic activity of fumagillin and some of its derivatives. *Antibiotics Ann* 1958-1959: 541-6.
29. Dezube BJ, Von Roenn JH, Holden-Wiltse J, et al. Fumagillin analog

in the treatment of Kaposi's sarcoma: a phase I AIDS Clinical Trial Group study. *J Clin Oncol* 1998;16:1444-9.

30. Liu S, Widom J, Kemp CW, Crews CM, Clardy J. Structure of human methionine aminopeptidase-2 complexed with fumagillin. *Science* 1998; 282:1324-7.
31. Griffith EC, Su Z, Niwayama S, Ramsay CA, Chang YH, Liu JO. Molecular recognition of angiogenesis inhibitors fumagillin and ovalicin by methionine aminopeptidase 2. *Proc Natl Acad Sci U S A* 1998;95:15183-8.
32. Katinka MD, Duprat S, Cornillot E, et al. Genome sequence and gene compaction of the eukaryote parasite *Encephalitozoon cuniculi*. *Nature* 2001;414:450-3.

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