Amphotericin B and Itraconazole for Treatment of Disseminated *Penicillium marneffei* Infection in Human Immunodeficiency Virus–Infected Patients

Thira Sirisanthana, Khuanchai Supparatpinyo, Joseph Perriens, and Kenrad E. Nelson

From the Department of Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand; Joint United Nations Programme on HIV/AIDS, Geneva, Switzerland; and Department of Epidemiology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland, USA

Disseminated infection with *Penicillium marneffei* is common in patients infected with human immunodeficiency virus (HIV) in Southeast Asia. Treatment with amphotericin B alone is effective but requires a prolonged hospital stay. We conducted an open-label nonrandomized study to evaluate the efficacy and safety of treatment with amphotericin B at a dosage of 0.6 mg/(kg · d) intraveneously for 2 weeks, followed by a 400-mg/d dosage of oral itraconazole for 10 weeks. Of the 74 HIV-infected patients we studied who had disseminated *P. marneffei* infection, diagnosed by positive fungal culture and clinical evidence of infection, 72 (97.3%) responded to the treatment. There were no serious adverse drug effects. It was concluded that the regimen was effective and safe for treatment of disseminated *P. marneffei* infection in HIV-infected patients.

Penicillium marneffei, the only dimorphic fungus of the genus *Penicillium,* is emerging as an important HIV-related pathogen in Southeast Asia [1]. Prior to the HIV disease epidemic, only a few human infections, mainly originating in Thailand and southern China, had been described [2, 3]. From June 1990 to August 1997, 1,115 cases of systemic mycosis due to *P. marneffei* were seen in HIV-infected patients at Chiang Mai University Hospital (Chiang Mai, Thailand) alone.

Infection with *P. marneffei* in HIV-infected patients is a serious systemic disease, with fever, anemia, weight loss, and skin lesions as the major manifestations [1]. The infection is associated with a high mortality rate if timely treatment with appropriate antifungal drugs is not given [1]. Drugs that have been used successfully to treat this infection include parenteral amphotericin B, given over the period of 6 weeks to 2 months [1–6]. However, in addition to the need for a prolonged hospital stay, amphotericin B frequently causes adverse drug effects [7].

The fungus is highly susceptible to itraconazole [8], but in one small study in which patients with disseminated *P. marneffei* infection were treated with itraconazole alone, the mean interval until fungal cultures became negative for those who were successfully treated was nearly 2 months [9]. In this study we evaluated the efficacy of treating disseminated *P. marneffei* infection with amphotericin B (0.6 mg/[kg · d]) for 2 weeks, followed by itraconazole (400 mg/d) taken orally for 10 weeks.

Received 7 October 1997; revised 5 January 1998.

Informed consent was obtained from the patients, and guidelines for human experimentation of the U.S. Department of Health and Human Services and of Chiang Mai University were followed.

Grant support: Janssen Research Foundation, Beerse, Belgium.

Reprints or correspondence: Dr. Thira Sirisanthana, Department of Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.

Clinical Infectious Diseases 1998;26:1107–10

1058–4838/98/2605–0016$03.00

Patients and Methods

Study Design

The study was approved by the institutional review board of Chiang Mai University and was carried out at Chiang Mai University Hospital between November 1993 and January 1996. All HIV-infected adults with disseminated *P. marneffei* infection who had given informed consent were included in this open-label, noncomparative trial. The diagnosis of HIV infection was made if the patient's serum was repeatedly reactive to HIV by both ELISA and a gel particle agglutination test. The diagnosis of *P. marneffei* infection was made by isolation of the organism from a clinical specimen (blood and/or skin biopsy specimen) from a patient with clinical evidence of infection.

Methods of isolation and mycological characteristics of *P. marneffei* have been described previously [10]. Patients were excluded if they were pregnant or breast-feeding; had a history of allergy to amphotericin B or azole antifungal agents; received treatment with systemic antifungal agents, i.e., amphotericin B, flucytosine, ketoconazole, fluconazole, or itraconazole during the month prior to enrollment; or received concurrent treatment with phenytoin, barbiturates, carbamazepine, rifampin, histamine-$_2$ receptor blockers, antacids, corticosteroids, cytotoxic chemotherapy, or investigational drugs.

The patients were given a 1-mg test dose of amphotericin B. A dosage of 0.3 mg/(kg · d) was then given if there had been no serious adverse reaction. A dosage of 0.6 mg/(kg · d) was used from the second to the 14th day. From the 15th day to the end of the 12th week, the patients were given two capsules of itraconazole twice daily. Each capsule contained

100 mg of the drug. All patients took trimethoprim-sulfamethoxazole as primary prophylaxis for *Pneumocystis carinii* infection.

Evaluation

Patients were evaluated daily when they were in the hospital. They were discharged at the end of the second week of treatment and were seen at the outpatient department every 2 weeks. The final evaluation was at the end of the 12th week. Each evaluation included assessment of clinical findings.

Culture of a biopsy specimen of a skin lesion, if present, was performed prior to treatment. A blood culture was performed, and a complete blood cell count and levels of serum creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and bilirubin were determined before treatment and at the end of the 2nd, 6th, 10th, and 12th week. A T-lymphocyte subset determination was made before treatment and at the end of the 12th week. Differences between baseline body weights and $CD4^+$ T-lymphocyte counts and those at the end of the 12th week were tested with Student's *t*-test for paired samples [11].

Response to antifungal treatment was defined as a negative blood culture for *P. marneffei* at the end of the 2nd, 6th, 10th, and 12th week as well as resolution of fever and disappearance of skin lesions.

Results

Seventy-six patients enrolled. Two were removed from the study in the 4th week because the diagnoses of *P. marneffei* infection were not confirmed by fungal culture. Of the 74 patients included in the analysis, 64 were male and 10 were female. Their ages ranged from 19 to 49 years, with a mean of 29.7 years.

Seventy-two patients acquired HIV infection from heterosexual contact, one acquired it from parenteral drug use, and the mode of acquisition by the remaining patient was unknown. None of the patients were receiving antiretroviral therapy at enrollment. None were given any antiretroviral drugs during the study. Blood cultures performed before treatment yielded *P. marneffei* for 65 patients (88%). For 43 patients, biopsy specimens of the skin lesions were cultured; 40 of these (93%) yielded *P. marneffei.*

The patients presented with systemic signs and symptoms of disseminated infection. These signs and symptoms were fever (71 of 74 patients, 96%), weight loss (96%), skin lesions (85%), generalized lymphadenopathy (85%), hepatomegaly (65%), and splenomegaly (23%). These clinical findings were similar to those described in an earlier study [1].

Compliance was assessed by comparing the number of itraconazole capsules returned at each visit with the number given at the previous visit. One-hundred percent compliance was observed at 344 of the 348 visits (98.8%) for which information was available. Evaluation at the end of the 12th week revealed that 72 patients (97.3%) responded to antifungal treatment.

One of the remaining two patients was lost to follow-up after the end of the 2nd week of treatment, and the other died after 10 weeks. He was a 28-year-old man whose fungemia cleared by the end of the 2nd week. After 4 weeks of treatment he was afebrile and had gained 4 kg. He died after a few days of illness, the main features of which were high fever and chills. An autopsy was not performed and the cause of death was not known.

By the end of the 2nd week, only 12 patients remained febrile and 11 patients still had skin lesions. By this time, fungemia had cleared in all 65 patients whose initial blood cultures were positive. At the end of the 4th week, no patient was febrile, and there was complete disappearance of the skin lesions in all patients. By the end of the 12th week, only two patients still had cervical lymphadenopathy, three had hepatomegaly, and none had splenomegaly. The 72 patients seen at the end of the study had gained an average of 3.3 kg (mean body weight at the 12th week, 49.6 kg [SD, 7.9]; mean baseline body weight, 46.3 kg [SD, 7.7]; $P = 1.31 \times 10^{-6}$).

The $CD4^+$ T-lymphocyte counts at the end of the 12th week (mean, 70.1/μL [SD, 62.6]) were not different from those obtained before treatment (mean, 63.5; SD, 47.5; $P = .33$).

Table 1 gives the data pertaining to laboratory abnormalities and adverse events at enrollment, during treatment, and at the end of the study. Many patients had abnormal laboratory values before the beginning of antifungal treatment. During the 12 weeks of treatment, the laboratory abnormality with the highest incidence (4.99 per 100 person-weeks) was anemia, followed by an increase in the values of aspartate aminotransferase (4.12), alkaline phosphatase (3.44), and alanine aminotransferase (2.84), and leukopenia (2.6). Six patients had an increased serum creatinine level, three developed thrombopheblitis, and two each had hypokalemia, rash, and chills.

No patient required any adjustment in the administration of amphotericin B or itraconazole because of the occurrence of laboratory abnormalities or adverse events. At the conclusion of the study, 14 patients still had anemia, and 16 had leukopenia; 18, 9, and 11 patients still had an elevated level of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase, respectively.

Discussion

Thailand has one of the most serious epidemics of HIV disease and AIDS. As of June 1997, a cumulative total of 64,594 cases of AIDS had been reported to the Ministry of Public Health; 23,522 (36%) of these occurred in the upper northern region of the country [12]. In this region, disseminated infection with *P. marneffei* is one of the most common HIV-related opportunistic infections, accounting for 14% of the initial clinical presentations of patients with AIDS [13]. Patients with disseminated *P. marneffei* infection had been successfully

Table 1. Laboratory abnormalities and adverse events at admission and during and at the end of the treatment for disseminated *Penicillium marneffei* infection in HIV-infected patients.

Variable	No. (%) of abnormalities at admission (n = 74)	No. of events per patients observed, during treatment	Person-time*	Incidence†	No. (%) of occurrences at week 12 (n = 72)
Laboratory abnormality					
Anemia	35 (47)	16/39	2,243	4.99	14 (19)
Leukopenia	16 (22)	16/58	4,302	2.60	16 (22)
Neutropenia	2 (3)	0/72	6,092	0.00	0
Thrombocytopenia	10 (14)	1/64	5,344	0.13	0
In value of					
Aspartate aminotransferase	34 (46)	15/40	2,547	4.12	18 (25)
Alanine aminotransferase	15 (20)	17/59	4,197	2.84	9 (12)
Alkaline Phosphatase	28 (38)	14/46	2,845	3.44	11 (15)
Bilirubin	3 (4)	2/71	5,928	0.24	0
Creatinine	0	6/74	5,787	0.73	0
Hypokalemia	0	2/74	6,106	0.23	0
Adverse event					
Thrombophlebitis	0	3/74	6,020	0.35	0
Rash	0	2/74	6,211	0.23	0
Chill	0	2/74	6,093	0.23	0
Nausea	0	1/74	6,181	0.11	0
Diarrhea	0	1/74	6,246	0.11	0

* Total person-days of follow-up.
† Incidence in 100 person-weeks.

treated with amphotericin B with or without flucytosine [1–6]. The total dosage of amphotericin B given was 1.4 g to >2 g over the period of 6 weeks to >2 months [2–6]. A treatment regimen that requires a shorter hospital stay would decrease the burden on the already overtaxed health care system.

Prolonged treatment with a high dosage of amphotericin B is also associated with anemia, decreased renal function, thrombophlebitis, hypokalemia, chills, fever, headache, nausea, vomiting, and malaise [7]. In a few cases, treatment with amphotericin B had to be discontinued because of these adverse drug effects [8, 14, 15]. Itraconazole, an orally active triazole antifungal agent, has relatively few adverse drug effects [16] and is highly active against *P. marneffei* in vitro [8]. However, one study in which patients were treated with 400 mg of itraconazole per day for 2 months, followed by 100 mg/d for 1 month, the mean interval between the start of treatment and sterility of the blood culture was 57 days [9].

Our treatment regimen of a short course of amphotericin B followed by itraconazole, given on an outpatient basis, was designed to decrease the length of the hospital stay and the cost of treatment. Other objectives were to lower the incidence of adverse drug effects associated with prolonged administration of amphotericin B and to achieve faster clearance of fungemia.

Seventy-two patients (97%) responded to the treatment. Fungemia cleared after 2 weeks in all 65 patients whose blood cultures were initially positive. In the remaining patients, resolution of fever, skin lesions, lymphadenopathy, hepatomegaly, and splenomegaly and an increase in weight provided evidence of response to therapy.

There has been little information on the optimal treatment for patients infected with *P. marneffei,* since not many cases have occurred outside Thailand. We reported our experience in treating our first 80 patients [8]. Among the 35 patients treated with amphotericin B in that retrospective study, 27 (77%) responded with clinical and microbiological resolution of infection; 9 of the 12 patients treated with itraconazole responded.

The better response rate in our present study may be due to the fact that diagnoses were made and treatment was begun earlier in this group of patients. Local physicians have become more familiar with the disease and patients seek medical care earlier. In addition, the response rate may have been better than that among patients treated with itraconazole alone because the regimen containing amphotericin B sterilized the blood faster.

Patients were infected with *P. marneffei* in the late stage of their HIV disease. The average $CD4^+$ T-lymphocyte count at enrollment was 63.5/μL. Many patients in our study had abnormal laboratory values at the beginning of the study. It is difficult to find out the exact cause of these abnormal values. They may be due to disseminated *P. marneffei* infection, other conditions associated with the late stage of HIV infection, or HIV infection itself. Patients also developed laboratory abnormalities during the study, and some still had abnormal values at the end of treatment.

It was not possible to unequivocally attribute these abnormalities to the effects of the administered drugs, specifically amphotericin B, itraconazole, and trimethoprim-sulfamethoxazole. However, an increased serum creatinine level, hypokalemia, thrombophlebitis, rash, chills, and nausea were not pres-

ent at the beginning of the treatment and were adverse effects commonly associated with amphotericin B [7]. In this study, none of these adverse reactions that were potentially caused by the treatment regimen were severe, and no adjustment in the administration of the drug was necessary.

Treatment with amphotericin B for 2 weeks, followed by itraconazole orally for 10 weeks, is safe and effective, and the regimen decreases the overall cost of treatment. It is recommended as the treatment regimen of choice for disseminated *P. marneffei* infection in HIV-infected patients. In one of our earlier studies [8], it was found that 12 of 40 patients who responded to initial therapy relapsed within 6 months. Thus, we recommend that just as for AIDS patients with cryptococcosis or histoplasmosis, secondary prophylaxis should be given to patients with *P. marneffei* infection who have responded to initial treatment. At the Chiang Mai University Hospital, the patients are given itraconazole (200 mg/d orally) as secondary prophylaxis for life.

Acknowledgments

The authors acknowledge Professor J. M. A. Lange (University of Amsterdam, the Netherlands); N. Tonkul (Janssen Research Council, Thailand); L. G. Dally (Istituto Superiore di Sanita, Rome); and S. Chariyalertsak, S. Kitiyawongs, W. Buranachokpaisal, T. Limpijarnkij, C. Khamwan, and J. Praparatanapan (Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand).

References

1. Supparatpinyo K, Khamwan C, Baosoung V, Nelson KE, Sirisanthana T. Disseminated *Penicillium marneffei* infection in Southeast Asia. Lancet **1994**;344:110–3.
2. Jayanetra P, Nitiyanant P, Ajello L, et al. Penicilliosis marneffei in Thailand: report of five human cases. Am J Trop Med Hyg **1984**;33:637–44.
3. Deng Z, Ribas JL, Gibson DW, Connor DH. Infection caused by *Penicillium marneffei* in China and Southeast Asia: review of eighteen published cases and report of four more Chinese cases. Rev Infect Dis **1988**;10:640–52.
4. Yuen WC, Chan YF, Loke SL, Seto WH, Poon GP, Wong KK. Chronic lymphadenopathy caused by *Penicillium marneffei:* a condition mimicking tuberculous lymphadenopathy. Br J Surg **1986**;73:1007–8.
5. Viviani MA, Tortorano AM, Rizzardini G, et al. Treatment and serological studies of an Italian case of penicilliosis marneffei contracted in Thailand by a drug addict infected with human immunodeficiency virus. Eur J Epidemiol **1993**;9:79–85.
6. Sekhon AS, Stein L, Garg AK, Black WA, Glezos JD, Wong C. Pulmonary penicillosis marneffei: report of the first imported case in Canada. Mycopathologia **1994**;128:3–7.
7. Bennett JE. Antimicrobial agents. In: Gilman AG, Rall TW, Nies AS, Taylor P, eds. The pharmacological basis of therapeutics. New York: Pergamon Press, **1991**:1165–81.
8. Supparatpinyo K, Nelson KE, Merz WG, et al. Response to antifungal therapy by human immunodeficiency virus infected patients with disseminated *Penicillium marneffei* infections and in vitro susceptibilities of isolates from clinical specimens. Antimicrob Agents Chemother **1993**;37:2407–11.
9. Supparatpinyo K, Chiewchanvit S, Hirunsri P, et al. An efficacy study of itraconazole in the treatment of itraconazole in the treatment of *Penicillium marneffei* infection. J Med Assoc Thai **1992**;75:688–91.
10. Supparatpinyo K, Chiewchanvit S, Hirunsri P, Uthammchai C, Nelson KE, Sirisanthana T. *Penicillium marneffei* infection in patients infected with human immunodeficiency virus. Clin Infect Dis **1992**;14:871–4.
11. Colton T. Inference on means. In: Colton T. Statistics in medicine. Boston: Little, Brown, **1974**:99–150.
12. Ministry of Public Health, Thailand. Summary of AIDS situation. Weekly Epidemiological Surveillance Report **1997**;28:S69–80.
13. Clezy K, Sirisanthana T, Sirisanthana V, Brew B, Cooper DA. Late manifestations of HIV in Asia and the Pacific. AIDS **1994**;8(suppl 2):S35–43.
14. So SY, Chau YF, Jones BM, et al. A case of invasive penicilliosis in Hong Kong with immunologic evaluation. Am Rev Respir Dis **1985**;131:662–5.
15. Peto TEA, Bull R, Millard PR, et al. Systemic mycosis due to *Penicillium marneffei* in a patient with antibody to human immunodeficiency virus. J Infect **1988**;16:285–90.
16. Philips P, Fetchick R, Weisman I, et al. Tolerance to and efficacy of itraconazole in treatment of systemic mycoses: preliminary results. Rev Infect Dis **1987**;9(suppl 1):S77–9.