Combination Therapy with Fluconazole and Flucytosine for Cryptococcal Meningitis in Ugandan Patients with AIDS

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We performed a randomized trial in which combination therapy with fluconazole and short-term flucytosine was compared with fluconazole monotherapy in 58 patients with AIDS-associated cryptococcal meningitis (CM). Thirty of these patients were randomized to receive combination therapy with fluconazole, 200 mg once a day for 2 months, and flucytosine, 150 mg/(kg · d) for the first 2 weeks, and 28 were randomized to receive monotherapy with fluconazole at the same dose for 2 months. Patients in both groups who survived for 2 months received fluconazole as maintenance therapy at a dose of 200 mg three times per week for 4 months. The combination therapy prevented death within 2 weeks and significantly increased the survival rate among these patients (32%) at 6 months over that among patients receiving monotherapy (12%) ($P = .022$). The combination therapy also resulted in a significant decrease in the severity of headache after 1 month of treatment, compared with monotherapy ($P = .005$). No serious adverse reactions were observed in patients receiving either regimen. These data indicate that treatment with fluconazole and short-term flucytosine is a cost-effective and safe regimen that improves the quality of life for patients with AIDS-associated CM in developing countries where human immunodeficiency virus is endemic.

Cryptococcal meningitis (CM) is the most common invasive deep fungal infection in patients with AIDS, occurring in 6%–10% of such patients in the United States and in ⩽30% in sub-Saharan Africa [1, 2]. This disease is regarded as a life-threatening opportunistic infection, particularly in Africa [3, 4]. The median survival time after the diagnosis of CM among patients with AIDS who do not receive antifungal chemotherapy is reported to be only 4 days in Malawi [3]. The high incidence of CM appears to be due to the prevalence of *Cryptococcus neoformans* in the domestic environments of patients with AIDS [5]. Treatment of CM with amphotericin B and flucytosine has been shown to be effective in these patients [6]. However, this treatment is difficult to manage in developing countries because of the toxicity of amphotericin B. Fluconazole, one of the recently developed triazole antifungal drugs, has shown a promising effect, comparable to that of intravenous amphotericin B, in the treatment of CM in patients with AIDS [7].

See the editorial response by Edwards and Edwards on page 1367.

Although treatment of CM with high-dose fluconazole or the combination of fluconazole and flucytosine has been tested in developed countries [8, 9], easily managed and cost-effective treatments are absolutely necessary for AIDS-associated CM in developing countries. We have previously reported the results of a preliminary study of oral fluconazole for the treatment of AIDS-associated CM in Uganda [10], which is one of the countries with the largest prevalence of HIV infection in the world [11]. The survival rates remained very low (30%) and were similar among patients receiving 200 mg or 400 mg of fluconazole for 2 months. To establish this easily managed and cost-effective therapy as a means of improving the quality of life for patients with AIDS and CM in developing countries, we designed the present study to compare the effects of treatment with low-dose fluconazole and short-term flucytosine with those of fluconazole therapy alone among such patients in Uganda. Herein we demonstrate that combination therapy was more beneficial than monotherapy for these patients.

Patients and Methods

Between January 1994 and May 1994, 58 patients >16 years of age who were found to be HIV seropositive by particle agglutination (Fuji Rebio, Tokyo) were enrolled at Mulago Hospital (Kampala, Uganda) by physicians of the Department of Medicine, Makerere Medical School (Kampala). Patients

Received 28 July 1997; revised 26 January 1998.

This work was presented in part at the 10th International Conference on AIDS held on 7–12 August 1994 in Yokohama, Japan.

Grant support: This work was supported in part by a grant from the Japanese Ministry of Education, Science, and Culture, and a grant from Pfizer Pharmaceuticals, Inc.

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Clinical Infectious Diseases 1998;26:1362–6

1058–4838/98/2606–0021$03.00

were enrolled if they had clinical symptoms of meningoencephalitis and a positive India ink test or CSF positive for cryptococcal antigen, as determined by latex agglutination (Eiken, Tokyo). CSF cultures, performed on Sabouraud agar, were positive for all enrolled patients. Patients were excluded if they were comatose or pregnant or if they had received any antifungal treatment during the previous month. The study protocol was reviewed and approved by the Ugandan National AIDS Research Subcommittee and the National Council of Science and Technology of Uganda.

Treatment

After written informed consent had been obtained from either the patient or the principal care giver, the patients were randomized to receive fluconazole monotherapy or combination therapy with fluconazole and flucytosine. Randomization was done by means of sealed envelopes. In the group receiving monotherapy, fluconazole was administered orally as a single daily dose of 200 mg for 2 months. The combination therapy consisted of oral fluconazole at a dose of 200 mg once daily for 2 months plus oral flucytosine at a dose of 150 mg/(kg · d), divided into three daily doses, for the first 2 weeks. Administration of flucytosine on a short-term basis may reduce cost as well as toxicity. Fluconazole was administered orally in a single, 400-mg loading dose only on the first day.

Fluconazole at a dose of 100 mg or 200 mg daily has been shown to be highly effective in the prevention of recurrent cryptococcal infection [12]. Thus, maintenance therapy with oral fluconazole at a dose of 200 mg three times per week for 4 months was administered to all patients in each group who successfully responded to primary therapy. When we diagnosed pulmonary tuberculosis in the enrolled patients, antituberculous drugs including rifampin, pyrazinamide, ethambutol, and isoniazid were also administered to these patients.

Evaluation

For evaluation at baseline, the clinical symptoms and signs were assessed and the CD4 and CD8 lymphocyte counts in the peripheral blood were determined by using the T4-8 kit (Dynal, Oslo) at the time of enrollment. The accuracy of CD4 and CD8 lymphocyte counts obtained by this method has been shown to be comparable to that of counts obtained by use of flow cytometry in African patients' blood samples [13]. After the baseline evaluation, patients were examined every week for the first 2 months and every 2 weeks thereafter until the 6-month study period was completed. At each visit, a physical examination was performed, and any adverse events that were judged to be related to treatment with fluconazole or flucytosine by the clinical investigators were assessed and recorded. Lumbar pucture was performed after primary therapy had been administered for 2 months and maintenance therapy had been administered for 4 months. CSF samples were assessed by India ink staining, cryptococcal antigen determination, and culture for *C. neoformans.*

Primary therapy was considered successful if the patients' conditions improved clinically or there was complete resolution of symptoms after 2 months of treatment. Maintenance therapy was initiated in these patients even if they had quiescent disease.

Antifungal Susceptibility Testing

In vitro susceptibility testing of 32 isolates of *C. neoformans* from patients with AIDS and CM was performed in Roswell Park Memorial Institute (RPMI) 1640 medium with L-glutamine and without sodium bicarbonate; the medium was buffered at pH 7.0 with 0.165 M MOPS (4-morpholinepropanesulfonic acid), according to the modified method of the National Committee for Clinical Laboratory Standards. Briefly, 100-μL volumes of the 2 $\times$ drug dilutions were suspended into sterile, flat-bottomed 96-well microplates [14]. One hundred microliters of yeast suspension were added to each well, resulting in the desired drug concentration and inoculum size of $1-5 \times 10^3$ cfu/mL. The MICs of fluconazole or flucytosine were defined as the lowest concentrations that resulted in a visual turbidity less than or equal to control (0.2 mL of growth control plus 0.8 mL of uninoculated RPMI 1640). The microplates were incubated at 35°C and read at 48 hours.

Statistical Analysis

Statistical comparisons of categorical clinical variables, including the severity of headache, were made by χ^2 analysis. Survival was defined as the interval from the date of enrollment to the date of death or the date of termination of the study at 6 months posttreatment, and differences in survival distribution were assessed by use of Cox's F test [15]. A P value of $<.05$ was considered significant. The initial target sample size of 65 was chosen to ensure that there would be at least 80% power to detect a difference of 30% (40% vs. 10%), with a one-sided alpha level of 0.05, in survival of patients at 6 months after treatment [16].

Results

Thirty of 58 patients with AIDS and CM were randomized to receive combination therapy with fluconazole and flucytosine, and 28 were randomized to receive fluconazole monotherapy (table 1). Before treatment, almost all patients (98%) had headaches, described as severe by 91%. Only 60% of the patients had fever, whereas 48% of the patients showed signs of decreased mentation, including somnolence (36%) and stupor (12%); 52% of the patients had normal sensorium. With respect to opportunistic infections other than CM, oral candidiasis was

Table 1. Clinical characteristics of patients with AIDS-associated cryptococcal meningitis in Uganda.

Characteristic	Fluconazole + flucytosine (n = 30)	Fluconazole alone (n = 28)	Total (n = 58)
Median age in y (range)	33.3 (24–53)	33 (23–50)	33.6 (23–53)
Male sex	14 (47)	11 (39)	25 (43)
Median body weight (kg)	46.9	51.5	49.0
Sign or symptom			
Headache	30 (100)	27 (96)	57 (98)
Severe	27 (90)	26 (93)	53 (91)
Moderate	3 (10)	1 (4)	4 (7)
Fever	17 (57)	18 (64)	35 (60)
Level of consciousness			
Stupor	3 (10)	4 (14)	7 (12)
Somnolence	13 (43)	8 (29)	21 (36)
Normal sensorium	14 (47)	16 (57)	30 (52)
Opportunistic infection			
Oral candidiasis	23 (77)	17 (61)	40 (69)
Pulmonary tuberculosis	2 (7)	3 (11)	5 (9)
Mean CD4 lymphocyte count (/mm^3)	80	73	77
Mean CD8 lymphocyte count (/mm^3)	227	188	208
Mean CD4/CD8 ratio	0.42	0.41	0.41

NOTE. Data are number (%) of patients unless otherwise indicated.

most frequently observed (69% of patients), and pulmonary tuberculosis was diagnosed in five (9%). Two patients with CM and three with pulmonary tuberculosis were randomized to receive combination therapy and monotherapy, respectively. The mean CD4 and CD8 peripheral-blood lymphocyte counts were 77/mm^3 and 208/mm^3, respectively, with a mean CD4/CD8 ratio of 0.41. There were no significant differences with respect to demographic characteristics, laboratory values, or clinical symptoms and signs of CM between the treatment groups at baseline.

We could evaluate the survival rate among only 50 of the patients after administration of primary and maintenance therapy for 6 months because during primary therapy, five patients (two receiving combination therapy and three receiving monotherapy) withdrew from the study, and during maintenance therapy, three patients (two receiving combination therapy and one receiving monotherapy) withdrew from the study (figure 1). Ten (40%) of 25 patients receiving fluconazole monotherapy died within the first 2 weeks of primary therapy, and only nine (36%) of these patients survived for 2 months. In contrast, the use of combination therapy reduced the death rate among these patients during the first 2 weeks (4 [16%] of 25 patients died), and 14 (56%) of 25 patients randomized to receive combination therapy survived during the full 2-month course of primary therapy. After primary and maintenance therapy for 6 months, survival rate among 25 patients (32%) receiving combination therapy was significantly higher than that (12%) among the 25 patients randomized to receive monotherapy (P = .022).

We also examined the severity of headache after 2 weeks, 1 month, and 2 months of combination therapy or monotherapy. After 1 month of treatment, patients who received combination therapy had significantly less-severe headaches than those who received monotherapy (table 2; P = .005), but this difference was not observed after 2 weeks or 2 months.

Despite the prolonged survival among patients who received combination therapy, the rate of positive CSF cryptococcal antigen cultures remained high after 2 months (12 [80%] of

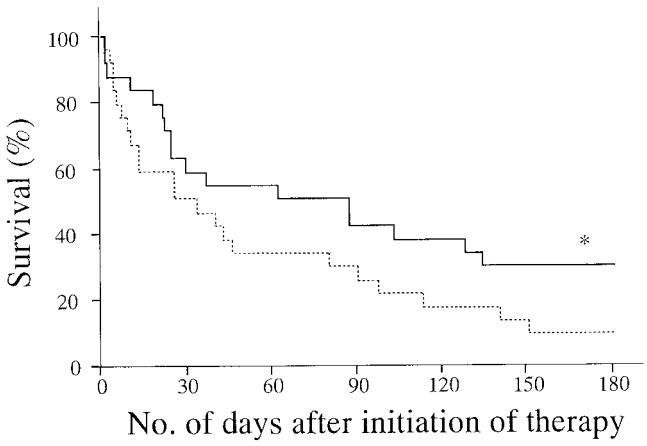

Figure 1. Survival curve for patients with AIDS and cryptococcal meningitis who received combination therapy with fluconazole and flucytosine (——) vs. fluconazole monotherapy (----). *P = .022, Cox's F test.

Table 2. Comparative analysis of the severity of headache in patients with AIDS-associated cryptococcal meningitis who received combination therapy or monotherapy.

Treatment	Duration of treatment (d)	No. of patients with indicated severity of headache				*P* value*
		None	Mild	Moderate	Severe	
Flu + 5-FC ($n = 30$)	0	0	0	3	27	.492
Flu alone ($n = 28$)		1	0	1	26	
Flu + 5-FC ($n = 24$)	14	5	10	4	5	.424
Flu alone ($n = 16$)		2	8	5	1	
Flu + 5-FC ($n = 18$)	30	11	6	1	0	.005
Flu alone ($n = 11$)		4	1	0	6	
Flu + 5-FC ($n = 15$)	60	13	2	0	0	.375
Flu alone ($n = 8$)		6	1	0	1	

NOTE. Flu = fluconazole; 5-FC = flucytosine.
* χ^2 test.

15) and at 6 months (5 [62.5%] of 8) after treatment. Similarly, four (50%) of eight patients who received monotherapy had culture-positive CSF after 2 months of treatment, but neither of two patients had culture-positive CSF after 6 months of treatment. These data indicate that a large proportion of the patients had quiescent disease at both endpoints of primary and maintenance therapy.

No serious adverse effects were noted in any of the enrolled patients during primary or maintenance therapy. Pulmonary tuberculosis was first detected in seven patients with AIDS-associated CM after enrollment. Three of these patients were receiving combination therapy (tuberculosis was detected in one during primary therapy and in two during maintenance therapy), and four of the patients were receiving monotherapy (tuberculosis was detected in all during primary therapy). A total of 12 patients with pulmonary tuberculosis were included in this study.

The results of in vitro susceptibility testing of antifungal agents have not regularly correlated with patients' clinical responses. However, recent papers have shown correlations between the MIC for *C. neoformans* and the minimum effective dose of fluconazole in mice [17, 18]. Therefore, we conducted in vitro susceptibility tests of fluconazole and flucytosine against 32 strains of *C. neoformans* that were isolated from the patients in this study. The MIC_{50} of fluconazole was 8.0 μg/mL, and the MIC_{90} was 16.0 μg/mL. However, the range of fluconazole MICs was broad (8–>128 μg/mL) because one isolate was highly resistant to this drug (MIC, >128 μg/mL). The range of flucytosine MICs was similarly broad (0.016–4 μg/mL). The MIC_{50} of flucytosine was 0.25 μg/mL, and the MIC_{90} was 1 μg/mL.

Discussion

In the present study we noted that headache was a prominent symptom among the patients (98%), a finding that was comparable to that for patients with AIDS and CM in Malawi (97%) and in the United States (67%) [3, 7]. The frequency of abnormal sensorium among our patients (48%) was comparable to that among patients with AIDS and CM in Malawi (58%) but appeared to be higher than that among such patients in the United States (27%). Similar rates of candidiasis (63%) and tuberculosis (17%) among AIDS patients with CM were observed in a previous study in Africa [4], since these diseases are associated with depletion of CD4 lymphocytes in the peripheral blood.

Previous studies of treatment with oral fluconazole (200 mg/d) for patients with AIDS-associated CM showed that the survival rate after primary therapy was much lower in Uganda (30%) than in the United States (82%) [7, 10]. Differences in survival between the two studies appear to be due to delay in diagnosis and lack of supportive therapies for these patients. A preliminary study of combination therapy with fluconazole (400 mg/d) and flucytosine (150 mg/[kg · d]) for CM was conducted in patients with AIDS [9]. The Kaplan-Meier estimate of the rate of clinical success at the end of 10 weeks and the median time to negative CSF cultures were reported to be 63% and 23 days, respectively. These results were superior to those obtained with fluconazole or amphotericin B alone. In addition, fluconazole was well tolerated, while flucytosine caused gastrointestinal complaints and fatal thrombocytopenia in some patients because of the long median duration of therapy (43 days) in this study.

In the present study, combination therapy with fluconazole and flucytosine for AIDS-related CM produced a significant increase in the number of patients who survived for 6 months and lessened the severity of headache after 1 month. Combination treatment not only prolonged survival but also improved the quality of life by lessening the severity of headache, the most debilitating symptom of CM. However, the rate of long-term survival after combination therapy remained low (32%). These beneficial effects of combination therapy are apparently

attributable to the addition of flucytosine to the regimen for the first 2 weeks. A recent paper documented a significant reduction in the burden of fluconazole-resistant *C. neoformans* in the brains of mice with CM that received combination therapy with fluconazole and flucytosine vs. fluconazole alone [18]. These data strongly support the beneficial effects of combination therapy observed in the present study even though we could not find significant differences in fungal culture results with combination therapy vs. monotherapy. Quantitative, rather than qualitative, culture methods should be used to demonstrate the difference in fungal culture results with combination therapy vs. monotherapy. Furthermore, it is noteworthy that no adverse effects were observed in the present study because flucytosine (150 mg/[kg · d]) was given only for 2 weeks.

Since the clinical effectiveness of daily fluconazole doses of 200 mg and 400 mg were similar [9], safety and cost-effectiveness considerations led us to adopt a fluconazole dosage of 200 mg per day for both monotherapy and combination therapy in this study. This dosage, however, might have been too low in view of the fungistatic activity of fluconazole, as CSF concentrations after administration of fluconazole at a dose of 400 mg have been reported to be 2.7–8.2 μg/mL [4]. The MICs of fluconazole for the isolates of *C. neoformans* tested in this study appeared to be higher than could be achieved with 200 mg of fluconazole. Poor penetration of fluconazole into CSF and reduced susceptibility of *C. neoformans* to fluconazole might have resulted in the quiescent disease in a large number of the patients in the present study.

The prevalence of tuberculosis among HIV-infected individuals in Uganda has been described previously [19]. Pulmonary tuberculosis was diagnosed in five of our cases before antifungal therapy was administered and in seven cases during antifungal therapy. Laroche et al. [4] have similarly reported a high incidence of tuberculosis (17%) among patients with AIDS-associated CM. This finding suggests the need for antituberculous prophylaxis during antifungal therapy among patients with AIDS-associated CM. It is notable, however, that rifampin causes a reduction of the half-life of fluconazole that may influence the antifungal activity of fluconazole against *C. neoformans* [20].

In summary, we demonstrated a significant reduction in the severity of headache after 1 month of treatment and a significant prolongation of survival after 6 months of treatment with fluconazole and short-term flucytosine among patients with AIDS-associated CM. Further clinical investigations are required to evaluate the role of combination therapy in the treatment of CM in patients with AIDS in developing countries where HIV is endemic.

Acknowledgments

The authors thank H. Miwa and M. Shimada for their assistance in performing the statistical analysis, and P. Ombasi and M. Akiyose for their technical assistance, and D. W. Nielsen and M. Pollack for their helpful comments on this manuscript.

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