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Response to Fluconazole by 23 Patients with Human Immunodeficiency Virus Infection and Oral Candidiasis: Pharmacological and Mycological Factors

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The MICs of fluconazole for strains of *Candida* species and the levels of fluconazole in serum were determined at day 0 and day 14 for 23 human immunodeficiency virus-infected patients with oral candidiasis who were treated orally with 100 mg of fluconazole per day for 14 days. Among the 23 patients, 11 (48%) were not clinically cured and had persistent isolation of *Candida albicans* ($n = 10$) and/or presence of non-*C. albicans* ($n = 6$). Clinical response could be predicted by the susceptibility of the strain to fluconazole determined at day 0. All 12 patients who were cured were infected with a strain for which the MIC was <0.78 mg/liter. All four patients who were infected with a strain for which the MIC was >3.12 mg/liter experienced clinical failure. These data suggest that a *C. albicans* strain could be defined as being susceptible when the MIC of fluconazole is <0.78 mg/liter and as being resistant when the MIC is >3.12 mg/liter.

Oropharyngeal candidiasis is the most frequent opportunistic infection in patients infected with the human immunodeficiency virus (HIV) and occurs in up to 95% of such patients (8). Fluconazole is now commonly used to treat this complication because of its demonstrated efficacy and low rate of toxicity (3, 9). Recently, the therapeutic failure of fluconazole has been reported despite the use of increasingly large doses (11). Clinical resistance has been related to the selection of strains of *Candida albicans* that are less susceptible to fluconazole and to the emergence of naturally resistant *Candida* species, such as *C. kruzei* and *C. glabrata* (2). Correlation of clinical results with the MICs of antifungal agents is difficult (13). Some studies have assessed in vitro susceptibility of the *Candida* strains to antifungal agents and suggested their correlation with clinical course (4, 15); one such study was designed to prospectively establish this correlation (7). We conducted a prospective study to further assess the relationship between the mycological and pharmacological data and the clinical response of oral candidiasis to fluconazole in HIV-infected patients.

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From December 1993 to April 1994, 28 HIV-infected patients with clinically symptomatic oral candidiasis, treated orally with a single daily dose (100 mg) of fluconazole for 14 days, were prospectively studied. At the end of the 14-day treatment, the patients were classified in three groups according to the clinical response of oral candidiasis. The first group included patients who were clinically cured, i.e., they had complete resolution of oral lesions and related symptoms. For the second group, improvement was defined as the partial resolution of symptoms and a decrease in the extent of oral lesions. In the third group, failure was defined as persistence of oral lesions and related symptoms. Strains of *Candida* species were isolated and identified from oral specimens taken from each patient at day 0 and day 14. The MIC of fluconazole was determined by the broth macrodilution method, according to National Committee for Clinical Laboratory Standards guidelines (10), at day 0 and day 14. Levels of fluconazole in serum were measured by high-performance liquid chromatography at day 14, just before and 2 h after oral administration of the daily dose. Expected peak and trough levels were 15 and 6 mg/liter, respectively.

Of the 28 consecutive patients included in the study, 5 were lost to follow-up before the day 14 evaluation. The 23 evaluable patients were 20 men and 3 women, and the mean age was 38 years (range, 26 to 50). The mean CD4 count was 75 cells per mm^3 (range, 2 to 368). At the end of the treatment, 12 patients had been cured (52%), 4 had improved (17%), and 7 had failed (31%) (Table 1). The mean CD4 count was higher in the group of patients who were cured (96 cells per mm^3) than in the two other groups (13.5 cells and 16 cells per mm^3, respectively). The mean number of previous fluconazole courses was higher in each of the groups who were not cured, i.e., 6.4 in the group that failed and 2.5 in the group that improved compared with 1.3 in the group that was cured.

At day 0, *C. albicans* was isolated from the 23 patients, and in 4 of 23 (17%) patients, illness was associated with a non-*C. albicans* strain (Table 1). MICs of fluconazole ranged from 0.1 to 6.25 mg/liter for *C. albicans* strains. It was 0.1 mg/liter for one *C. parapsilosis* strain and it was >6.25 mg/liter for three non-*C. albicans* strains (one strain each of *C. glabrata*, *C. kruzei*, and *C. inconspicua*). All 12 patients who were cured were infected with a *C. albicans* strain for which the MIC was <0.78 mg/liter. Among the four patients who experienced improvement, one patient was infected both with a *C. albicans* strain, for which the MIC was 0.2 mg/liter, and with a *C. parapsilosis* strain. The three other patients were infected with a *C. albicans* strain for which the MIC was 3.12 mg/liter. Among the 7 patients who failed, four were infected with a *C. albicans* strain for which the MIC was 6.25 mg/liter (one patient was coinfected with a *C. inconspicua* strain), two were

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TABLE 1. MICs of fluconazole for *Candida* strains at day 0 according to clinical response after a 14-day course of fluconazole in 23 HIV-infected patients with oral candidiasis

Clinical response	Total no. of strains	No. of patients infected with strains for which the MICs (mg/liter) were as follows:							
		0.1	0.2	0.39	0.78	1.56	3.12	6.25	>6.25
Cured (n = 12)									
C. albicans	12	5	4	3	0	0	0	0	0
Non-*C. albicans*	0								
Improved (n = 4)									
C. albicans	4	0	1	0	0	0	3	0	0
Non-*C. albicans*	1	1							
Failed (n = 7)									
C. albicans	7	0	1	0	0	2	0	4	0
Non-*C. albicans*	3	0	0	0	0	0	0	0	3

infected with a *C. albicans* strain for which the MIC was 1.56 mg/liter (one patient was coinfected with a *C. glabrata* strain), and one was infected with a *C. albicans* strain for which the MIC was 0.20 mg/liter (this patient was also coinfected with a *C. kruzei* strain). Thus, the rate of cure was 86% for the patients infected with a *C. albicans* strain for which the MIC was <0.78 mg/liter. This rate of cure was significantly higher than that for patients infected with a *C. albicans* strain for which the MIC was ≥0.78 mg/liter. The rate of failure was 100% for patients infected with a *C. albicans* strain for which the MIC was >3.12 mg/liter. For the patients who were infected with a *C. albicans* strain for which the MIC was ≥1.56 but ≤3.12 mg/liter, the rate of failure was 40% (2 of 5) (Fig. 1). One of the two patients infected with a *C. albicans* strain for which the MIC was <0.78 mg/liter and who were not cured was coinfected with a *C. krusei* strain for which the MIC was 100 mg/liter.

At the end of the 14-day treatment, *C. albicans* was isolated from 11 of 23 (48%) patients. One of the patients was from the group that was cured, and 10 of the patients were from the two other groups. Concomitant colonization with six non-*C. albicans* strains was observed in 5 of these 11 patients (45%). One symptomatic patient was infected only with *C. kruzei*. The

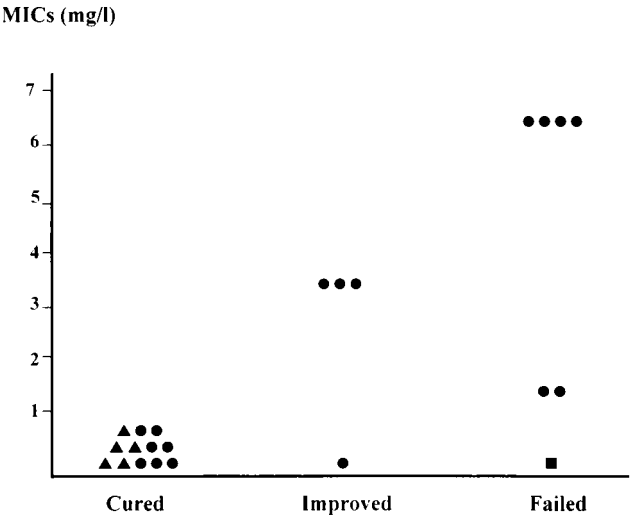

FIG. 1. MICs (mg/liter) of fluconazole for the *C. albicans* strains isolated at day 0, according to clinical evolution (cured, improved, or failed) after 14-day course of fluconazole. Each point represents one patient with the corresponding strain. Symbols indicate number of CD4 cells per mm^3: ▲, >100; ■, ≥50 to ≤100; ●, <50.

MICs of fluconazole for these *C. albicans* strains were <0.78 (n = 2), 1.56 (n = 1), 3.12 (n = 5), and 6.25 mg/liter (n = 3). Thus, from day 0 to day 14, the number of *C. albicans* strains for which the MIC was <0.78 mg/liter had decreased from 14 to 2, while the number of *C. albicans* strains for which the MIC was ≥0.78 mg/liter remained unchanged at 9. None of the MICs for the *C. albicans* strains increased significantly between day 0 and day 14.

Peak and trough levels of fluconazole in serum did not differ according to the type of clinical response. These levels were, respectively, 6.4 ± 3.4 mg/liter (n = 5) and 4.6 ± 1.6 mg/liter (n = 6) for patients who were cured, 3.1 mg/liter (n = 1) and 1.7 ± 0.1 mg/liter (n = 2) for patients who improved, and 6.1 ± 4.4 mg/liter (n = 5) and 3.1 ± 2.1 mg/liter (n = 3) for patients who failed.

C. albicans remains the major species responsible for oropharyngeal candidiasis (6). The incidence of recurrence, increasing with the progression of the underlying immunodeficiency, contributes to weight loss, which is a significant prognostic factor for HIV infection. The incomplete efficacy of treatment regimens to eradicate oropharyngeal carriage has led to the emergence of resistant strains and consequent clinical failure. Several authors have retrospectively studied factors which could influence the emergence of resistance. Clinical failure has been related to advanced HIV infection, to a low CD4 count, and to the number of previous courses of fluconazole (1, 14, 15). In fact, a single 14-day course of fluconazole favored both the persistence of *C. albicans* strains for which the MICs were ≥1.56 mg/liter and the emergence of non-*C. albicans* strains with reduced susceptibility to fluconazole, while *C. albicans* disappeared in almost all patients who were cured. It did not appear in this study that a lower level of fluconazole in serum was associated with a less favorable clinical response, although overall drug levels in serum were lower than expected. However, the number of patients for whom the level of fluconazole in serum was determined was small and may have been insufficient to detect such a relationship. This study also provides additional data regarding the correlation between in vitro antifungal susceptibility testing of the initial strain and therapeutic outcome (4, 14, 15). The proportion of patients in this study who were cured was significantly higher when the MIC of fluconazole was <0.78 mg/liter. Indeed, a MIC of fluconazole of <0.78 mg/liter had a prognostic significance for cure of 80%. These data lead us to propose susceptibility breakpoints as follows: a strain can be considered susceptible when the MIC is <0.78 mg/liter, intermediate when the MIC is between 0.78 and 3.12 mg/liter, and resistant when the MIC is >3.12 mg/liter. Another factor associated with clinical failure, despite *C. albicans* susceptibility, is the emer-

gence of non-*C. albicans* strains which are naturally less susceptible to fluconazole (5, 11, 12). The increasing frequency of colonization both with *C. albicans* strains with a reduced susceptibility to fluconazole and with non-*C. albicans* strains is probably related to the widespread use of azoles for maintenance therapy (5). Whether new strategies might help to minimize the development of resistant *C. albicans*, such as the use of discontinued and alternative azole derivatives, should be further investigated.

Meanwhile, for patients with CD4 counts lower than 50 cells per mm^3 or for cases of recurrent oral candidiasis treated with fluconazole, determination of the MIC for the strain isolated before treatment is begun could be recommended. It could also be useful for epidemiological purposes and for assessment of compliance.

REFERENCES

1. **Baily, G. G., F. M. Perry, D. W. Denning, and B. K. Mandal.** 1994. Fluconazole-resistant candidosis in an HIV cohort. AIDS **8:**787–792.
2. **Boken, D. J., S. Swindells, and M. G. Rinaldi.** 1993. Fluconazole-resistant *Candida albicans*. Clin. Infect. Dis. **17:**1018–1021.
3. **British Society for Antimicrobial Chemotherapy Working Party.** 1992. Antifungal chemotherapy in patients with acquired immunodeficiency syndrome. Lancet **340:**648–651.
4. **Cameron, M. L., W. A. Schell, S. Bruch, J. A. Bartlett, H. A. Waskin, and J. R. Perfect.** 1993. Correlation of in vitro fluconazole resistance of *Candida* isolates in relation to therapy and symptoms of individuals seropositive for human immunodeficiency virus type 1. Antimicrob. Agents Chemother. **37:** 2449–2453.
5. **Fan-Havrad, P., D. Capano, S. M. Smith, A. Mangia, and R. H. K. Eng.** 1991. Development of resistance in *Candida* isolates from patients receiving prolonged antifungal therapy. Antimicrob. Agents Chemother. **35:**2302–2305.
6. **Franker, C. K., F. M. Lucartorto, B. S. Johnson, and J. J. Jacobson.** 1990. Characterization of the mycoflora from oral mucosal surfaces of some HIV infected patients. Oral Surg. Oral Med. Oral Pathol. **69:**685–687.
7. **Galgiani, J. N.** 1990. Susceptibility of *Candida albicans* and other yeasts to fluconazole: relation between *in vitro* and *in vivo* studies. Rev. Infect. Dis. **12**(Suppl. 3)**:**272–275.
8. **Gazzard, B. G., and D. Smith.** 1990. Oral candidosis in HIV-infected patients. Br. J. Clin. Pract. **44**(Suppl. 71)**:**103–108.
9. **Hay, R. J.** 1990. Overview of studies of fluconazole in oropharyngeal candidiasis. Rev. Infect. Dis. **12**(Suppl. 3)**:**334–337.
10. **National Committee for Clinical Laboratory Standards.** 1992. Reference method for broth dilution antifungal susceptibility testing of yeasts. Proposed standard MP27-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
11. **Newman, S. L., T. P. Flanigan, A. Fisher, M. G. Rinaldi, M. Stein, and K. Vigilante.** 1994. Clinically significant mucosal candidiasis resistant to fluconazole treatment in patients with AIDS. Clin. Infect. Dis. **19:**684–686.
12. **Powderly, W. G.** 1992. Mucosal candidiasis caused by non-albicans species of *Candida* in HIV-positive patients. AIDS **6:**604–605.
13. **Rex, J. H., M. G. Rinaldi, and M. A. Pfaller.** 1995. Resistance of *Candida* species to fluconazole. Antimicrob. Agents Chemother. **39:**1–8.
14. **Ruhnke, M., A. Eigler, I. Tennagen, B. Geiseler, E. Engelmenn, and M. Trautmann.** 1994. Emergence of fluconazole-resistant strains of *Candida albicans* in patients with recurrent oropharyngeal candidiasis and human immunodeficiency virus infection. J. Clin. Microbiol. **32:**2092–2098.
15. **Troillet, N., C. Durussel, J. Bille, M. P. Glauser, and J. P. Chave.** 1993. Correlation between in vitro susceptibility of *Candida albicans* and fluconazole-resistant oropharyngeal candidiasis in HIV-infected patients. Eur. J. Clin. Microbiol. Infect. Dis. **12:**911–915.