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Azole Resistance in Oropharyngeal *Candida albicans* Strains Isolated from Patients Infected with Human Immunodeficiency Virus

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For 212 oropharyngeal isolates of *Candida albicans*, the fluconazole MICs for 50 and 90% of strains tested were 0.5 and 16 μg/ml, respectively, and those of itraconazole were 0.05 and 0.2 μg/ml, respectively. Of 16 isolates for which fluconazole MICs were >64 μg/ml, itraconazole MICs for 14 were ≤0.8 μg/ml and for 2 were >6.4 μg/ml. Most fluconazole-resistant strains remained susceptible to itraconazole; whether itraconazole will prove effective for refractory thrush remains to be shown.

Oropharyngeal candidiasis is the most common fungal infection in patients with AIDS (7). Although candidiasis is usually amenable to therapy with local or systemic antifungal drugs, failures of fluconazole therapy for mucocutaneous infections due to *Candida albicans* have been reported (1, 3, 4, 8, 11, 15, 17–20). Itraconazole has been shown to be efficacious for thrush (22) but has been used infrequently. With increasing reports of failure of fluconazole therapy, we sought to determine the itraconazole susceptibilities of fluconazole-susceptible and -resistant *C. albicans* isolated from our patients infected with human immunodeficiency virus (HIV).

***C. albicans* strains.** A total of 212 isolates of *C. albicans* from 46 different patients were studied. These strains had been isolated over the course of 2 1/2 years (July 1991 to January 1994) from patients with HIV infection who were treated in the Infectious Diseases Clinic at the Ann Arbor Veterans Affairs Medical Center. Thirty-four patients had at least one episode of thrush, and 12 patients were colonized with *C. albicans* but did not have thrush. Some, but not all, of these patients had taken part in a previously reported study (20).

Susceptibility testing. The proposed standard broth macrodilution method for antifungal susceptibility testing of fluconazole (National Committee for Clinical Laboratory Standards document M27-P) was used for in vitro susceptibility tests (16). Stock solutions were stored at −70°C after preparation in distilled water for fluconazole and in dimethylsulfoxide for itraconazole. Each day an assay was performed, a new vial of stock solution was thawed and diluted in distilled water. For fluconazole, final concentrations ranged from 0.06 to 64 μg/ml; for itraconazole, final concentrations ranged from 0.006 to 6.4 μg/ml. Turbidity in each tube was assessed by the criteria put forth by Espinel-Ingroff et al., in which the MIC was defined as the tube with ≥80% inhibition of growth (6, 16). For each set of susceptibility studies, three standard organisms (a *C. krusei* strain and two *C. albicans* strains) were included.

For all 212 isolates, the fluconazole MIC for 50% of strains tested (MIC_{50}) was 0.5 μg/ml and the MIC_{90} was 16 μg/ml; the itraconazole MIC_{50} was 0.05 μg/ml, and the MIC_{90} was 0.2 μg/ml. The fluconazole MICs for the majority of isolates (151) were ≤2 μg/ml; for these isolates, the itraconazole MIC_{50} and MIC_{90} were correspondingly low, at 0.05 and 0.1 μg/ml, respectively. For those 61 isolates for which fluconazole MICs ranged from 4 to >64 μg/ml, the fluconazole MIC_{50} was 8 μg/ml and the MIC_{90} was 64 μg/ml, while the corresponding itraconazole MIC_{50} was 0.2 μg/ml and the MIC_{90} was 0.4 μg/ml. Thus, the itraconazole MIC_{50}s and MIC_{90}s increased fourfold for those isolates for which fluconazole MICs were increased. However, the increase in fluconazole MICs was more dramatic (Fig. 1). For those 16 isolates for which fluconazole MICs were >64 μg/ml, the itraconazole MICs were 0.1 μg/ml (2 isolates), 0.2 μg/ml (7 isolates), 0.4 μg/ml (4 isolates), and 0.8 μg/ml (1 isolate); the MIC was >6.4 μg/ml for only 2 isolates.

Nine patients had 61 isolates for which fluconazole MICs were ≥4 μg/ml. Eight of the nine had been treated with fluconazole for over 1 year. For two of these eight, initial and follow-up care was given elsewhere, and one of the eight patients died within 2 weeks of being seen for the first time. All three patients had thrush while taking 200 mg of fluconazole daily. After 1 1/2 years of fluconazole therapy, one patient's thrush cleared only after amphotericin B was given. The fluconazole MICs were from 4 to 16 μg/ml, while the itraconazole MICs were 0.05 μg/ml for two patients (six isolates over 2 months) and 0.2 μg/ml in the remaining patient (five isolates over 4 months). None were treated with itraconazole.

Five patients were treated in our clinic over 2 years. All had CD4 counts of <50/μl and had been on fluconazole prophylaxis (100 mg thrice weekly) or had been treated with 100 mg daily for at least 1 year. The MICs for the isolates from one patient demonstrated a change from 0.2 to 32 μg/ml (total of eight isolates tested) after 2 years, and this patient's thrush cleared on 200 mg of fluconazole daily. The other four had organisms for which fluconazole MICs increased from 0.2 to >64 μg/ml over 2 years; in these patients, 800 mg of fluconazole daily failed to achieve a clinical response. The itraconazole MICs increased from 0.05 to 0.4 μg/ml in two patients and from 0.05 to >6.4 μg/ml in the other two patients (total of 52 isolates tested over 2 years) (Fig. 2). In three patients, 200-mg itraconazole capsules given daily produced no clinical response, and two of the three ultimately responded to intravenous amphotericin B therapy. As noted previously, karyotyping by contour-clamped homogeneous electric field electrophoresis demonstrated that resistance developed in the original colonizing strain in two patients and a new resistant strain was acquired in the third patient (20).

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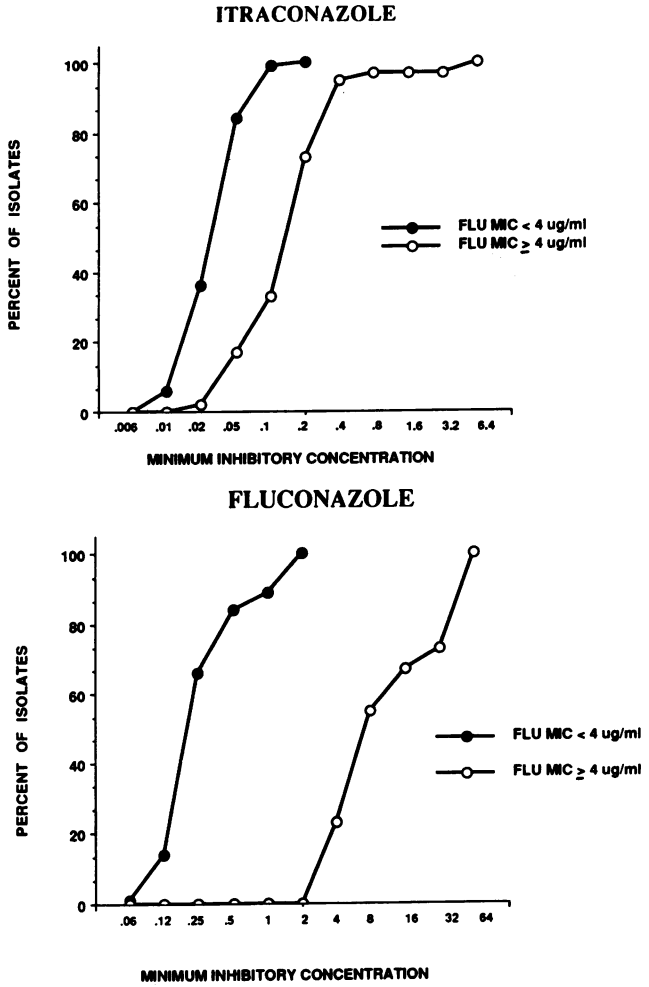

FIG. 1. Percentage of isolates at each MIC of itraconazole (top) and fluconazole (bottom). The isolates are separated into those for which fluconazole MICs were <4 μg/ml and those for which fluconazole MICs were ≥4 μg/ml. Most, but not all, isolates for which fluconazole MICs were high maintained low itraconazole MICs.

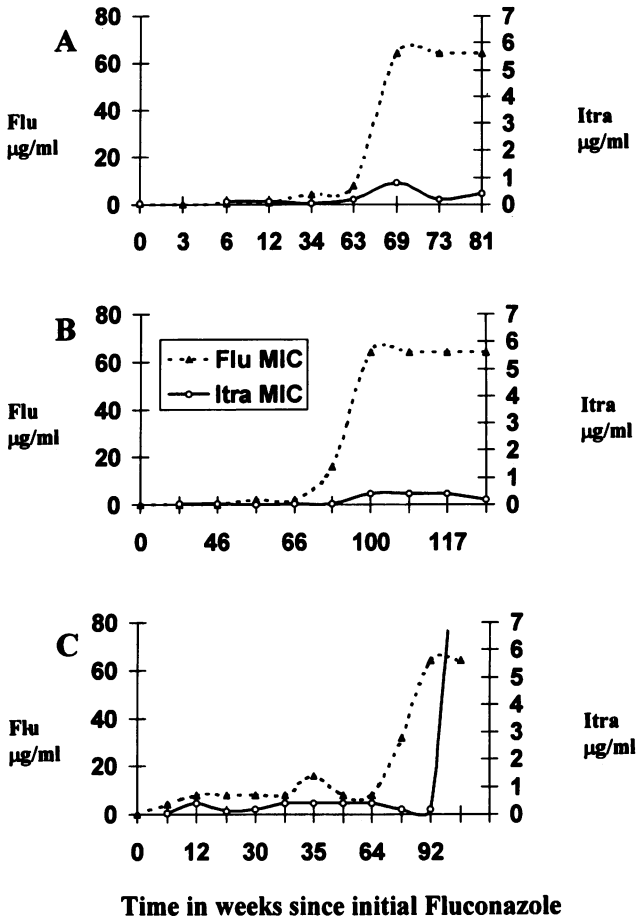

FIG. 2. Representative time course of changes in fluconazole and itraconazole MICs for three patients (panels A, B, and C, respectively). By the end of the time period shown for each patient, neither 800-mg fluconazole tablets nor 200-mg itraconazole capsules cleared their oropharyngeal candidiasis.

The remaining patient, who has been described previously (20), differs in that he appeared to have acquired a strain for which the fluconazole MIC was 16 μg/ml from his sexual partner prior to his ever receiving fluconazole and before his CD4 count fell below 200/μl. The itraconazole MIC for this isolate was 0.2 μg/ml. The patient failed to respond to 200 mg of fluconazole daily, but after loss of this particular strain, subsequent episodes of thrush responded to 200 mg of fluconazole daily.

Fluconazole failure, manifested by poor clinical response and increasing MICs for *C. albicans* in vitro, has been reported from several different countries (1, 3, 4, 8, 11, 15, 17–20). In almost every instance, failure has occurred in patients who have been on fluconazole for long periods of time. When noted, these patients have had CD4 counts of <20/μl (20). By monitoring individual patients over time, several laboratories have noted increasing fluconazole MICs associated with clinical failure (1, 15, 17, 18, 20).

By using karyotyping techniques, several laboratories, including our own, have noted that an existing strain appears to develop resistance to fluconazole over time (1, 15, 17, 18, 20). However, in other patients, failure is due to colonization with a newly acquired strain (1, 20).

National Committee for Clinical Laboratory Standards proposed standards for fluconazole susceptibility testing were adapted for use with itraconazole. No standards currently exist for in vitro susceptibility testing with itraconazole (9). Although it now appears that in vitro susceptibility results correlate with clinical failure of fluconazole (1, 18, 20), it is not known if the same is true for itraconazole.

In this study, we have shown that isolates of *C. albicans* for which the fluconazole MIC is >64 μg/ml show an upward shift in itraconazole MICs, but the change is not nearly as pronounced as that noted with fluconazole. Whether itraconazole will be useful in those patients who no longer respond to fluconazole is not known (18). For three of our patients, therapy with itraconazole capsules was not successful.

Serum itraconazole levels above 0.4 μg/ml should be attainable in most persons. However, poor absorption of both ketoconazole and itraconazole due to achlorhydria has been noted in patients with HIV infection (2, 13, 14, 21). Thus, the failure of itraconazole to successfully treat thrush in this population may be related not to resistance of the organism to the drug but to poor absorption. Further studies using new formulations of itraconazole (10) or combining itraconazole

with acidifying agents to increase absorption in patients with advanced HIV infection and recalcitrant thrush should be carried out (5, 12).

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