Emergence of Resistance to Fluconazole as a Cause of Failure during Treatment of Histoplasmosis in Patients with Acquired Immunodeficiency Disease Syndrome

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In sequential clinical trials of treatment for histoplasmosis in patients with acquired immunodeficiency syndrome, therapy with fluconazole failed in a higher proportion of patients than did therapy with itraconazole. To determine the cause for failure with fluconazole, antifungal susceptibility testing that used modified National Committee on Clinical Laboratory Standards procedures was performed on all baseline and failure isolates. Failure occurred more frequently in patients with baseline isolates with fluconazole minimum inhibitory concentrations (MICs) \( \geq 5 \mu g/mL \) versus lower MICs; 29% versus 3%, respectively. There was at least a 4-fold increase in fluconazole MIC in the isolates from 10 (59%) of 17 patients for whom paired pretreatment and failure or relapse isolates were available. Cross-resistance to itraconazole was not seen. In conclusion, fluconazole is less active than itraconazole for \( H. capsulatum \) and induces resistance during therapy, which accounted for treatment failure in some patients.

Fluconazole appears to be somewhat less effective than itraconazole as a treatment for histoplasmosis in patients with AIDS. As initial treatment in patients with mild or moderately severe histoplasmosis, the response to fluconazole and itraconazole was similar, 74% versus 85%, respectively [1, 2]. Fungemia cleared more rapidly with itraconazole, however. As maintenance therapy, <5% of patients relapsed while taking itraconazole [3], whereas 31% relapsed taking fluconazole [2]. Also, fluconazole proved to be less effective than itraconazole in an animal model of histoplasmosis [4]. Fluconazole yields higher blood levels than itraconazole; its lower efficacy may result from inferior activity against \( H. capsulatum \), from development of resistance, or both.

The occurrence of relapse after initial response to therapy supports a hypothesis that \( H. capsulatum \) developed resistance to fluconazole during treatment, as was demonstrated in a single case reported elsewhere [5]. The MIC of that patient’s isolate increased from 0.62 \( \mu g/mL \) at initiation of therapy to 20 \( \mu g/mL \) at the time of failure 16 weeks later. The isolate remained highly susceptible to itraconazole. The purpose of this report is to examine the relationship of susceptibility
to fluconazole with response to treatment for histoplasmosis in patients with AIDS and the risk for development of cross-resistance to itraconazole.

**METHODS**

**Clinical trial.** Results of the trial evaluating fluconazole for treatment of mild to moderately severe cases of disseminated histoplasmosis in patients with AIDS have been reported elsewhere [2]. Patients were treated with fluconazole, 600 mg once daily for 8 weeks, as induction therapy, followed by 200 mg daily for chronic maintenance treatment in version 1 (V1) of the protocol [2]. Interim analysis prompted amendment of the study to increase the induction phase dosage to 800 mg daily and extend the duration to 12 weeks and to increase the maintenance phase dosage to 400 mg daily in version 2 (V2). Eligibility criteria were identical in versions 1 and 2, however. Induction therapy failure was defined as failure of fever to resolve, signs and symptoms of histoplasmosis to improve, and positive cultures to clear, and “maintenance relapse” was defined as death or recurrent illness caused by histoplasmosis.

Fungal blood cultures were obtained at baseline and monthly during induction therapy, at the time of suspected relapse during maintenance therapy, and to follow up a positive blood culture at the last visit. Isolates were mailed to the Histoplasmosis Reference Laboratory in Indianapolis for antifungal susceptibility testing.

**Antifungal susceptibility testing.** Antifungal susceptibility testing was performed with use of slight modifications of National Committee for Clinical Laboratory Standards (NCCLS) standardized methods developed for yeasts [6]. Fluconazole was dissolved in dimethyl sulfoxide, and itraconazole was dissolved in polyethylene glycol (MW 300) and heated to 60°C for 1 h. Two-fold drug dilutions in RPMI-1640 medium were tested at concentrations between 0.31 and 160.0 \( \mu \text{g/mL} \) for fluconazole and 0.019 and 1.0 \( \mu \text{g/mL} \) for itraconazole. The NCCLS method was modified for determination of the *Histoplasma* MIC. First, the *Histoplasma* inoculum was standardized by comparison to McFarland standard of 5 at 530 nm, then diluted 1:100, and the *Candida parapsilosis* ATCC 90018 control was prepared according to the NCCLS method, by comparison to a 0.5 McFarland standard, then diluted 1:2000. This modification was required because of the slower growth rate of *H. capsulatum*. The second modification of the NCCLS protocol was prolongation of the incubation time for *Histoplasma* to 96–120 h of incubation at 37°C, again on the basis of the slower growth rate of *H. capsulatum*. Growth of *H. capsulatum* was scored by comparison to controls grown without the presence of drug. Inhibition of at least 80% compared with the no-drug control was defined as the MIC.

**Statistical methods.** The distribution of the MICs to fluconazole and itraconazole were highly skewed. Nonparametric statistical techniques were used to summarize and analyze the data. The median, minimum, and maximum MICs are reported. The Wilcoxon rank-sum test was used to test for differences in the median MICs between the nonresponders and responders, between the 2 protocol versions, and between the 2 antifungals. A sign test was used to test for a change in the median MIC from baseline to failure or relapse in those subjects who did not respond to treatment. Fisher’s exact test was used to test for the significance of the frequency of baseline MIC \( \geq 5.0 \mu \text{g/mL} \) in those who responded versus those with treatment failure or relapse.

**RESULTS**

**Comparison of susceptibility of baseline isolates with outcome of therapy.** In the group of 65 subjects for whom baseline isolates were available, 37 responded to treatment and 28 did not respond. Of these 28 nonresponders, 10 represented induction failures and 18 represented maintenance relapses. Combin-
ing the results of baseline isolates for V1 (n = 24) and V2 (n = 41) cases, median MIC to fluconazole was significantly lower in those subjects who responded (n = 37) than in those who did not respond (n = 28) to treatment, 0.62 (range, 0.31–5.0) versus 1.25 µg/mL (range, 0.62–10.0), respectively (P = .0068, figure 1). Baseline fluconazole MICs ≥5.0 µg/mL were more frequent in the patients with subsequent treatment failure or relapse (8 [29%] of 28 patients) than in the responders (1 [3%] of 37 patients), P = .0038.

MICs of baseline isolates to fluconazole were significantly higher than to itraconazole. The median MIC to fluconazole was 1.25 µg/mL (range, 0.31–10.0), compared with 0.019 (range, 0.019–0.077) for itraconazole (P < .0001). The MIC required for inhibition of 90% of isolates studied (MIC90) was 5.0 µg/mL for fluconazole and 0.038 µg/mL for itraconazole.

Reproducibility was examined by comparison of MICs obtained on 2 separate occasions. MICs to fluconazole were within a 2-tube dilution range in 20 (95.2%) of 21 isolates tested in this analysis. Reproducibility to itraconazole was examined in 28 isolates, and all were within the 2-tube range of acceptable reproducibility for tube dilution assays.

**Change in susceptibilities comparing baseline and induction failure or maintenance relapse isolates.** Isolates available in 17 pairs from enrollment (baseline) and at induction therapy failure (n = 5) or maintenance therapy relapse (n = 12) were tested in the same assay, to eliminate interassay variability. At least a 4-fold increase in MIC occurred in isolates from 10 (59%) of the 17 patients (figure 2). A ≥4-fold increase occurred in 4 of 9 V1 patients and 6 of 8 V2 patients. No increase in MIC to itraconazole was observed. The median MIC to fluconazole increased from 1.25 µg/mL at baseline to 10 µg/mL at failure or relapse (P = .0042), whereas the itraconazole MICs remained unchanged, 0.019 µg/mL at baseline versus 0.019 µg/mL at failure or relapse, P = 1.0.

**DISCUSSION**

Information from cases from both versions 1 and 2 of the study was combined to provide sufficient data for this analysis. The dosage and duration of treatment was greater in V2 (800 mg/day for 12 weeks) than V1 (400 mg/day for 8 weeks), which potentially biased the analysis. However, despite the differences in intensity of treatment, the clinical outcome was very similar. Fifty percent of the V1 failed (10 of 20 patients), because of either no response to induction therapy (n = 4) or relapse during maintenance therapy (n = 6). In V2, 24 (49%) of 49 patients had failure during induction (n = 13) or maintenance (n = 11) therapy. Furthermore, the baseline MIC findings and changes during treatment in the patients with failure or relapse were similar in both versions of the study. Thus, this analysis does not appear to have been compromised by combining the data from the 2 versions of the study.

This is the first description of the use of the NCCLS method for measurement of the susceptibility of a large number of clinical isolates of *H. capsulatum* to antifungal agents. The NCCLS method required minor modifications because of the slow growth rate of *H. capsulatum*. This procedure was highly reproducible, with MICs within a 2-tube variation in 95% of isolates for fluconazole and 100% for itraconazole. These data show that outcome of fluconazole therapy correlated with in vitro susceptibility to fluconazole, which supports findings in a murine model [4].

Reduced susceptibility of *H. capsulatum* to fluconazole, compared with itraconazole, in part explains the inferiority of fluconazole for therapy of histoplasmosis. The median MIC of isolates obtained before therapy was 66 times higher to fluconazole than to itraconazole, 1.25 versus 0.019 µg/mL, respectively. Even considering the higher drug concentrations achieved with fluconazole than itraconazole, fluconazole still remains less active. For example, the average blood concentration of fluconazole in patients treated with 800 mg daily was 44.6 µg/mL in the fluconazole study [2], compared with ~6.8
μg/mL with itraconazole, 400 mg daily [1], and the ratio of blood concentration to MIC greatly favored itraconazole, 35:1 for fluconazole versus 358:1 for itraconazole. For failure isolates, the median MIC was 10.0 μg/mL to fluconazole versus 0.019 μg/mL to itraconazole, and differences in ratios of drug level to MIC were even more striking, 4:1 versus 358:1, respectively.

In addition to the higher baseline MIC to fluconazole than to itraconazole, development of resistance during therapy further impacted fluconazole’s effectiveness for treatment of histoplasmosis in this population. At least a 4-fold increase in MIC in 59% of patients with failure or relapse during therapy supports this hypothesis. Although the analysis was complicated by amendment of the study, which created 2 different treatment groups, and reduction in dosage during maintenance phase of treatment, the findings in these subgroups were similar to those of the merged groups, which supports this analysis and the conclusions of the study. Other evaluations of the role of susceptibility to fluconazole in the treatment of cryptococcal meningitis failed to show any improved antifungal effect by increasing the concentration of fluconazole, which supports this analysis of the combined dosages [7].

Concern exists about the potential for development of resistance to antifungal agents as they are used chronically or intermittently and the potential for induction of cross-resistance to other antifungal agents. Cross-resistance to itraconazole has been demonstrated among strains of C. albicans tance to other antifungal agents. Cross-resistance to itraconazole intermittently and the potential for induction of cross-resistance as they are used chronically or

References