NOTES

Development of Fluconazole Resistance in Candida albicans Causing Disseminated Infection in a Patient Undergoing Marrow Transplantation

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Oral candidiasis due to azole-resistant Candida albicans is an increasing problem in patients with AIDS who receive prolonged periods of fluconazole prophylaxis. Infection with C. albicans is also frequent in patients undergoing transplantation. However, azole resistance has not been appreciated as a major problem for these patients, presumably because they receive a relatively short duration of fluconazole prophylaxis. We describe a case of disseminated candidiasis due to fluconazole-resistant C. albicans in a patient following marrow transplantation. Restriction fragment length polymorphism analysis with use of the C. albicans strain-specific Ca3 probe was performed on sequential isolates. Identical banding patterns were obtained, thereby confirming that a fluconazole-susceptible endogenous C. albicans acquired azole resistance during a brief exposure to the drug and subsequently caused disseminated infection. This observation raises questions regarding the incidence, significance, and mechanism of azole resistance in fungi causing infection in this population.

Infections caused by Candida are frequent following marrow transplantation, occurring in up to 11% of patients [1]. Infection due to azole-resistant Candida albicans, which has emerged as a problem in patients with AIDS who receive prolonged periods of prophylactic fluconazole, has recently been described in patients with cancer [2, 3]. The lack of resistance in C. albicans causing infection in this population is presumed to be due to the short duration of exposure to fluconazole.

We report a case of disseminated infection with azole-resistant C. albicans that developed in a marrow transplant patient during fluconazole prophylaxis. DNA typing of a series of sequential isolates by means of restriction fragment length polymorphism (RFLP) analysis revealed that one strain of C. albicans acquired resistance after only 23 days of fluconazole exposure.

Case Report

A 30-year-old man with T cell non-Hodgkin’s lymphoma received an unrelated, partially HLA (human leukocyte antigens)-mismatched marrow transplant in 1994. Lymphoma was diagnosed in 1993, and subsequently, he underwent six cycles of chemotherapy with cyclophosphamide, vincristine sulfate, prednisone, and doxorubicin, which resulted in complete remission. He did not receive any antifungal medications. In November 1993, marrow examination revealed 15% lymphoid infiltration, and he was referred to our institution for transplantation.

He was treated with cyclophosphamide, antithymocyte globulin, and total body irradiation before infusion of marrow. Prophylaxis with oral fluconazole (400 mg/d) was begun on 31 January 1994, 8 days before transplantation (day 0). On day +6, fever prompted discontinuation of fluconazole therapy and fluconazole prophylaxis. DNA typing of a series of sequential isolates by means of restriction fragment length polymorphism (RFLP) analysis revealed that one strain of C. albicans acquired resistance after only 23 days of fluconazole exposure.

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Materials and Methods

Rectal swab specimens for cultures were taken before and during fluconazole prophylaxis, and blood specimens for cultures were obtained during febrile episodes (temperature, >38.3°C). Samples were plated on Sabouraud dextrose agar and incubated for 48 hours at 37°C. Individual colonies of *C. albicans* were identified by germ tube testing and stored frozen at -70°C in 10% glycerol. Susceptibility testing for fluconazole was performed by the broth microdilution method at the Fungus Testing Laboratory, University of Texas Health Sciences Center at San Antonio [4]. Resistance was defined as an MIC of fluconazole of >8 μg/mL [5].

RFLP analysis with use of the *C. albicans*-specific probe Ca3 was used to determine the genetic relatedness of the nine isolates [6]. Genomic DNA was isolated after cell shearing with glass beads [7]. DNA was digested with the restriction enzymes *Eco*RI, *Bgl*II, *Bcl*I, and *Bam*HI. Southern blotting was performed [8]. Filters were hybridized at 60°C with the Ca3 probe labeled by means of random priming, washed, and exposed to x-ray film [8].

Results

Four rectal isolates (1–4), four blood isolates (5–8), and one postmortem lung isolate (9) of *C. albicans* were obtained. The first isolate was obtained 1 day before the initiation of fluconazole prophylaxis. Isolates 2 and 3 were obtained while the patient was receiving oral fluconazole therapy. Isolates 4 through 6 were obtained while the patient was receiving intravenous fluconazole therapy. Isolates 7 through 9 were obtained after fluconazole therapy had been discontinued. The susceptibilities of the isolates to fluconazole (MIC), the sites of culture specimens, the dates that the specimens were obtained, and the antifungal regimen are shown in figure 1. After 14 days of fluconazole administration, the susceptible isolates were becoming resistant (MIC, 8 μg/mL). The MICs for the blood isolates were the highest (32–64 μg/mL). The lung isolate was susceptible to fluconazole (MIC, 1 μg/mL).

RFLP analysis with use of the restriction enzyme *Eco*RI and the Ca3 probe revealed identical banding patterns, thereby verifying that all isolates represented the same strain of *C. albicans* (figure 2). The patterns of the *Bcl*I, *Bam*HI, and *Bgl*II digests were also identical (data not shown). Thus, after 23 days of fluconazole administration, a single azole-susceptible colonizing strain of *C. albicans* became fluconazole-resistant and caused disseminated infection.
Discussion

The series of nine isolates from our patient represents one strain of *C. albicans* that acquired resistance to fluconazole, in varying amounts, over time. The methods we used to determine fluconazole susceptibility and the genetic relatedness of *Candida* have been accepted and proven to be reliable. The MICs were determined in a reference laboratory. RFLP analysis with use of the Ca3 probe has been used extensively for molecular typing of *C. albicans* [6, 9, 10].

Fluconazole-resistant *C. albicans* has been reported to cause oropharyngeal candidiasis in patients with AIDS worldwide [5, 11, 12]. In these cases, resistance develops when susceptible endogenous strains of *C. albicans* acquire resistance or are replaced by other strains that are already resistant. The development of resistance is associated with repeated or continuous exposure to low dosages (50–200 mg/d) of fluconazole [5, 11, 13].

It is not surprising that azole resistance has now become a problem for other immunocompromised populations, since fluconazole is being used more often as prophylaxis. Nolte et al. [3] recently described two patients with cancer who developed disseminated infection with azole-resistant *C. albicans*. Because of the short duration of drug exposure in these patients, the investigators concluded that an already resistant strain emerged because of the selection pressure of fluconazole. Unfortunately, colonizing strains were not available for analysis in their study. In our study, one susceptible strain became resistant after only 23 days of fluconazole administration.

In our series, isolate 9, obtained from lung tissue at autopsy (7 days after the discontinuation of fluconazole therapy) was susceptible (MIC, 1 μg/mL). It is possible that the same colonizing strain of *C. albicans* present in the rectum was also present in the respiratory tract but did not become resistant to fluconazole because it was protected from exposure to the same quantities of drug. Alternatively, it is possible that both susceptible and resistant isolates of the same strain colonized this patient and that fluconazole had a selection effect, thus increasing the likelihood of recovering a resistant isolate during therapy. Finally, the isolate may have lost resistance after fluconazole therapy was discontinued. We are currently studying the mechanisms of resistance in this series of isolates to understand this observation.

It is now apparent that the development of fluconazole resistance in *Candida* infecting the HIV-infected population is not a limited phenomenon. This finding, along with the increased use of fluconazole prophylaxis, emphasizes our need to define the mechanisms involved in the development of drug resistance. Further studies should also address the epidemiology of candidiasis in patients with cancer and transplant recipients so we can further refine our use of fluconazole as antifungal prophylaxis.

References