CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Nov. 1999, p. 921–923 Vol. 6, No. 6
1071-412X/99/$04.00+0

Successful Treatment of Fluconazole-Resistant Oropharyngeal Candidiasis by a Combination of Fluconazole and Terbinafine

MAHMOUD A. GHANNOUM* AND BONI ELEWSKI

Center for Medical Mycology and Mycology Reference Laboratory, University Hospitals of Cleveland, and Case Western Reserve University, Cleveland, Ohio

Received 20 May 1999/Returned for modification 21 June 1999/Accepted 18 August 1999

Increasing incidence of resistance to conventional antifungal therapy has demanded that novel therapies be introduced. Recent in vitro studies have shown that combinations involving azoles and allylamines may be effective in inhibiting fluconazole-resistant fungi. In this report, we describe the case of a 39-year-old woman who presented with white patches on her buccal mucosa, tongue, and palate with a bright erythematous erosive base. A fungal culture revealed *Candida albicans*. The patient failed to respond to the initially prescribed fluconazole therapy. Failure of therapy can be attributed to a developed resistance to fluconazole from the patient's intermittent use of this antifungal agent at varying dosages for the preceding 2 years due to a diagnosis of onychomycosis. In vitro testing of the culture from the patient showed elevated MICs of fluconazole, itraconzole, and terbinafine (MICs were 32, 0.5, and 64 μg/ml, respectively). Our goal was to combine therapies of fluconazole and terbinafine in an attempt to clear the fungal infection. Impressively, this combination resulted in the clearing of the clinical symptoms and the patient has successfully been asymptomatic for more than 12 months posttreatment.

The approvals of the azoles and the triazoles in late 1980s and early 1990s were major advances in our ability to treat safely and effectively local and systemic fungal infections. The highly safe profile of triazoles, in particular, fluconazole, led to extensive use. Fluconazole has been used to treat in excess of 16 million patients, including over 400,000 AIDS patients. Concomitant with this widespread use, there have been increasing reports of fluconazole-resistant *Candida albicans* strains, many of which are cross-resistant to other antimycotics (for a review, see the work of Ghannoum and Rice [6]). The likelihood of resistance development and patients becoming refractory increases when these patients are on azoles for an extended period (13). Alternative therapies for the treatment of resistant *Candida* isolates are currently being sought. Combining fluconazole with other antifungal agents has been suggested as an approach to achieve synergy and broaden the spectrum of activity to include fluconazole-resistant fungi (12). Use of terbinafine in combination with azoles has been suggested as a potential therapeutic option (16). Terbinafine is a clinical antimycotic which was first introduced in 1991 and is now marketed worldwide in both oral and topical formulations primarily for the treatment of fungal infections of the skin, nails, and hair. In humans, terbinafine is well absorbed after oral administration and maximal concentrations in plasma are reached within 2 h following oral administration. It binds strongly to plasma proteins, with more than 90% of proteins being bound. Terbinafine is extensively metabolized, and 15 metabolites have been identified (17). Importantly, it has been shown recently that terbinafine displays potent synergy with fluconazole in vitro against azole-resistant *Candida* strains (5, 8, 15). In spite of these encouraging in vitro data demonstrating synergy against fluconazole-resistant *Candida*, use of terbinafine and fluconazole clinically to treat patients with oropharyngeal candidiasis who are refractory to fluconazole has not been reported. This report describes the successful treatment with a combination therapy of terbinafine plus fluconazole of a patient with oropharyngeal candidiasis who failed to respond to fluconazole.

* Corresponding author. Mailing address: Center for Medical Mycology, Department of Dermatology, 11100 Euclid Ave., LKS 5028, Cleveland, OH 44106-5028. Phone: (216) 844-8580. Fax: (216) 844-1076. E-mail: mag3@po.cwru.edu.

CASE REPORT

A 39-year-old woman with fibromyalgia and no history of tobacco use, xerostomia, or diabetes mellitus was referred to us for evaluation of a painful tongue that failed to respond to oral fluconazole (200 mg daily) used for over 2 weeks in August 1997. Because of the failure to respond to fluconazole, the clinical diagnosis of oral candidiasis was questioned. Her past history is pertinent in that she had been on fluticasone propionate (a glucocorticoid nasal spray [Flonase], 0.05%; Glaxo Wellcome Inc., Research Triangle Park, N.C.) for over 1 year prior to the development of oral candidiasis. Other medications taken daily included cimetidine (Tagamet, histamine H_2-receptor antagonist), diclofenac sodium (Voltaren, nonsteroidal antiinflammatory agent), and desipramine hydrochloride (Norpramin, antidepressant drug). Additionally, this patient had used antibiotics intermittently for a variety of other medical problems, including sinusitus and upper respiratory tract infection. She had also been taking fluconazole intermittently for the previous 2 years due to a diagnosis of onychomycosis. She had taken one dosage of fluconazole (100 mg daily) for more than 6 months, and then her dosage was changed to 400 mg once weekly.

She presented with white patches on her buccal mucosa, tongue, and palate with a bright erythematous erosive base that was KOH positive for fungal elements. A fungal culture revealed *C. albicans*. Testing for susceptibility to fluconazole, itraconazole, and terbinafine was performed with this isolate. Our data showed that the MICs of fluconazole, itraconazole, and terbinafine, for the isolated strain were elevated, being 32.0, 0.5, and 64.0, respectively. Based on published data suggesting synergy between azoles and terbinafine, the patient was treated with fluconazole (200 mg per day) plus terbinafine (250 mg per day) for 2 weeks (15). Her condition totally cleared and

she was symptom free. She stopped using fluticasone propionate 5 months following the clearance of any symptoms indicative of oropharyngeal candidiasis. Treatment with fluticasone propionate did not affect the oropharyngeal flora of the patient, as evidenced by a positive fungal culture. A follow-up visit showed that the patient was asymptomatic even 12 months posttreatment.

MATERIALS AND METHODS

Isolation and identification. To isolate the causative organism, the patient's oral cavity was swabbed and the organism was cultured on Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) by surface spreading. Next, the petri dish was incubated at 35°C for 48 h. Following growth, the yeast was identified as *C. albicans* by a germ tube test and with the API 20C identification system (bioMerieux SA, Marcy l'Etoile, France).

Antifungal agents. Fluconazole powder was obtained from Pfizer Pharmaceuticals Group (New York, N.Y.), while terbinafine was supplied by Novartis Research Institute (Vienna, Austria). Fresh stock solutions (1 mg/ml) of both antifungals were prepared according to the manufacturers' recommendations.

Determination of MIC. Susceptibility testing was performed by a broth microdilution assay as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) document M27-A (10). This method uses RPMI 1640 medium buffered at pH 7, with 2×10^3 to 5×10^3 cells as an inoculum and with 48 h and 35°C as the incubation time and temperature, respectively. For all three antifungal agents, the endpoint was defined as the lowest concentration of drug that caused at least 80% growth inhibition compared to the level of growth in the control well. *Candida krusei* ATCC 6258 was used as a reference quality-control strain during the time the assay was performed. The MIC (32 μg/ml) for this strain was within the range predicted for fluconazole (16 to 64 μg/ml) by NCCLS document M27-A.

RESULTS AND DISCUSSION

The patient described in this case report was referred to us for treatment of oropharyngeal candidiasis which was not responding to fluconazole. A number of factors may have contributed to the presence of oral candidiasis, including the use of steroid inhalers, as well as a history of intermittent use of antibiotics (see above). Dennis and Itkin (3) reported that 5 of 25 patients treated with steroid inhalers developed oropharyngeal candidiasis, and Zegarelli and Kutscher (22) reported similar cases for patients using triamcinolone aerosol. Seelig has extensively reviewed the role of antibiotics in the development of candidiasis (18–20). It is widely accepted that treatment with broad-spectrum antibiotics is likely to lead to *Candida* overgrowth (11). Therefore, use of fluticasone propionate and intermittent antibiotic use may have been the underlying predisposing factors for oropharyngeal candidiasis in this patient. Due to the lack of available data, it is not possible to associate oral candidiasis in this patient with the use of antidepressants and antiinflammatory agents.

We report for the first time the successful treatment of a patient who had a fluconazole-refractory oropharyngeal candidiasis with a combination of fluconazole and terbinafine. Failure of this patient to respond to fluconazole treatment could be attributed to the low dose of fluconazole initially used (100 mg/day) to treat the patient. This conclusion is based on information that supports the contention that *Candida*'s response to fluconazole is dose dependent (14). This dependence on dose is particularly true when the MIC for the isolated strain is high, as is the case with the candidal isolate obtained from our patient. In their analysis of interpretive breakpoints for antifungal susceptibility testing of *Candida* and triazoles, the members of the NCCLS subcommittee on antifungal agents reported that the success rate for patients treated with 100 mg of fluconazole per day for strains for which MICs are $\leq$8 μg/ml is >90% but that the success rate for patients treated for isolates for which MICs are $\geq$8 μg/ml is slightly less because of a reduced ability of the patients to respond to fluconazole therapy (14).

The general principle of combined therapy of azoles and allylamines has been shown in two examples of successful treatment of fungal infections with terbinafine and itraconazole: one case of mycetoma caused by *Madurella mycetomatis* (4) and another case of cutaneous *Scopulariopsis brevicaulis* infection (2). More-extensive data demonstrating synergy between azole and terbinafine come from in vitro studies. Terbinafine showed in vitro synergy with the azoles fluconazole, itraconazole, and miconazole in *Cryptococcus neoformans* (9) and with fluconazole and itraconazole against four *Aspergillus fumigatus* strains (16). Other studies with *Trypanosoma cruzi*, the agent of Chagas disease, showed in vitro synergy between terbinafine and ketoconazole (7, 21). Combination of terbinafine and the triazole ICI 153,066 resulted in a fungicidal action against *C. albicans*, although the drugs were fungistatic when they were used singly (1).

The observed synergy between fluconazole and terbinafine is not surprising, because mechanistically, these two agents inhibit different steps of the same pathway, namely, the ergosterol biosynthesis pathway. Barrett-Bee and Ryder (1) provided evidence that the simultaneous accumulation of squalene (as a result of terbinafine action) and 14-methylsterols (as a result of azole action) occurs in cases of *Candida* treated with terbinafine plus an azole.

Our findings suggest that the use of fluconazole plus terbinafine provides a possible therapeutic option for the treatment of fluconazole-refractory candidiasis.

ACKNOWLEDGMENTS

We thank Heather Norris and Nancy Isham for their technical assistance.

REFERENCES

1. **Barrett-Bee, K., and N. S. Ryder.** 1992. Biochemical aspects of ergosterol biosynthesis inhibition, p. 410–436. *In* J. A. Sutcliffe and N. H. Georgopapadakou (ed.), Emerging targets for antibacterial and antifungal chemotherapy. Chapman and Hall, New York, N.Y.
2. **Cox, N. H., and B. Irving.** 1993. Cutaneous 'ringworm' lesions of *Scopulariopsis brevicaulis*. Br. J. Dermatol. **129:**726–728.
3. **Dennis, M., and I. H. Itkin.** 1964. Effectiveness and complications of aerosol dexamethasone phosphate in severe asthma. J. Allergy **35:**70–76.
4. **Engelkens, H. J. H., and B. Naafs.** 1994. Mycetoma. Br. J. Dermatol. **131:** 722–723.
5. **Fothergill, A. W., I. Leitner, J. G. Meingassner, N. S. Ryder, and M. G. Rinaldi.** 1996. Combination antifungal susceptibility testing of terbinafine and the triazoles fluconazole and itraconazole, abstr. E53, p. 91. *In* Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
6. **Ghannoum, M. A., and L. B. Rice.** Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clin. Microbiol. Rev., in press.
7. **Lazardi, K., J. A. Urbina, and W. De Souza.** 1990. Ultrastructural alterations induced by two ergosterol biosynthesis inhibitors, ketoconazole and terbinafine, on epimastigotes and amastigotes of *Trypanosoma* (*Schizotrypanum*) *cruzi*. Antimicrob. Agents Chemother. **34:**2097–2105.
8. **Leitner, I., J. G. Meingassner, and N. S. Ryder.** 1996. Potential of terbinafine for combination with other antimycotics against *Candida* and *Aspergillus*, abstr. 472. *In* Abstracts of Clinical Dermatology 2000.
9. **Mikami, Y., K. Yazawa, J. Uno, and K. Nishimura.** 1991. Comparison of antifungal activity of amphotericin B, miconazole, itraconazole, flucytosine and fluconazole against clinically isolated *Cryptococcus neoformans* by MIC and IC_{50} values, and their combination effects. Chemotherapy (Tokyo) **39:** 1–8.
10. **National Committee for Clinical Laboratory Standards.** 1997. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard M27-A. National Committee for Clinical Laboratory Standards, Wayne, Pa.
11. **Odds, F. C.** 1988. Iatrogenic factors that predispose to candidiasis, p. 104–108. *In Candida* and candidosis, 2nd ed. Bailliere Tindall, London, United Kingdom.
12. **Polak, A.** 1996. Combination therapy for systemic mycosis. Infection **17:**203–209.
13. **Redding, S. J., J. Smith, G. Farinacci, M. Rinaldi, A. Fothergill, J. Rhine-Chalberg, and M. Pfaller.** 1994. Resistance of *Candida albicans* to flucon-

azole during treatment of oropharyngeal candidiasis in a patient with AIDS: documentation by *in vitro* susceptibility testing and DNA subtype analysis. Clin. Infect. Dis. **18:**240–247.

14. **Rex, J. H., M. A. Pfaller, J. N. Galgiani, M. S. Bartlett, A. Espinel-Ingroff, M. A. Ghannoum, et al.** 1997. Development of interpretive breakpoints for antifungal susceptibility testing: conceptual framework and analysis of *in vitro-in vivo* correlation data for fluconazole, itraconazole and *Candida* infections. Clin. Infect. Dis. **24:**235–247.
15. **Rodero, L., R. Vitale, F. Hochenfaller, C. Canteros, and G. Davel.** 1996. *In vitro* activity of terbinafine in combination with triazole drugs against fluconazole resistant *Candida* species, abstr. E56, p. 91. *In* Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
16. **Ryder, N. S., and I. Leitner.** 1996. Activity of terbinafine against *Aspergillus in vitro*, in combination with amphotericin B or triazoles, abstr. E54, p. 91. *In* Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
17. **Ryder, N. S., and B. Favre.** 1997. Pharmacokinetics of terbinafine. Rev. Contemp. Pharmacother. **8:**289–297.
18. **Seelig, M. S.** 1966. Mechanisms by which antibiotics increase the incidence and severity of candidiasis and alter the immunological defenses. Bacteriol. Rev. **30:**442–459.
19. **Seelig, M. S.** 1966. The role of antibiotics in the pathogenesis of *Candida* infections. Am. J. Med. **40:**887–917.
20. **Seelig, M. S.** 1968. The rationale for preventing anti-bacterial-induced fungal overgrowth. Med. Times **96:**689–710.
21. **Urbina, J. A., K. Lazardi, T. Aguirre, M. M. Piras, and R. Piras.** 1988. Antiproliferative synergism of the allylamine SF 86-327 and ketoconazole on epimastigotes and amastigotes of *Trypanosoma* (*Schizotrypanum*) *cruzi*. Antimicrob. Agents Chemother. **32:**1237–1242.
22. **Zegarelli, E. V., and A. H. Kutscher.** 1964. Oral moniliasis following intraoral topical corticosteroid therapy. J. Oral Ther. **1:**304–307.