ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1988, p. 780–781
0066-4804/88/050780-03$02.00/0

Vol. 32, No. 5

MIC and Fungicidal Activity of Terbinafine against Clinical Isolates of *Aspergillus* spp.

H. J. SCHMITT,[1,2] E. M. BERNARD,[1] J. ANDRADE,[1] F. EDWARDS,[1] B. SCHMITT,[1] AND D. ARMSTRONG[1]*

Infectious Disease Service and Diagnostic Microbiology Laboratory, Department of Medicine and Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, New York 10021,[1] and Universitaetskinderklinik Mainz, Mainz, Federal Republic of Germany[2]

Received 7 December 1987/Accepted 9 February 1988

Terbinafine and amphotericin B MICs for 90% of strains tested were 1.6 and 0.4 μg/ml against *Aspergillus fumigatus* (16 strains), 0.8 and 3.2 μg/ml against *Aspergillus flavus* (10 strains), and 0.4 and 1.6 μg/ml against *Aspergillus niger* (10 strains), respectively. For all species tested, the minimal inhibitory and fungicidal concentrations for 90% of strains of both drugs were identical and the inoculum size did not have a major effect on the results.

Aspergillus species can cause life-threatening opportunistic infections among immunocompromised patients. Invasive aspergillosis is difficult to diagnose and often responds poorly to treatment. Amphotericin B remains the drug of choice despite its considerable toxicity.

Terbinafine belongs to a new class of antifungal agents (8). It is a synthetic naphthalenemethanamine that inhibits squalene epoxidase, a key enzyme in ergosterol biosynthesis of fungi (3, 5). Its mode of action is highly selective, i.e., it is much more inhibitory to fungal than to mammalian sterol biosynthesis (4). Terbinafine can be administered orally, and preliminary studies in humans indicate that it is well tolerated (7).

To evaluate the potential use of terbinafine in human aspergillosis, we compared its in vitro inhibitory and lethal activities against 36 clinical isolates of *Aspergillus* species with the activities of amphotericin B and ketoconazole.

(These data were presented in part at the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy, New York, N.Y., 4 to 7 October 1987 [H. J. Schmitt, E. M. Bernard, J. Andrade, F. Edwards, B. Schmitt, and D. Armstrong, abstr. no. 981].)

Source and preparation of antifungal agents. Amphotericin B (Fungizone) was obtained from E. R. Squibb & Sons, Princeton, N. J., and terbinafine was obtained from Sandoz Ltd., Basel, Switzerland. Amphotericin B was initially diluted in distilled water, and terbinafine was initially diluted in dimethyl sulfoxide. Ketoconazole was initially diluted in absolute ethanol. Further drug dilutions were made in distilled water at log 2 concentrations ranging from 0.025 to 51.2 μg/ml. Finally, 0.1-ml portions of each concentration were added to wells of microdilution plates.

Source and preparation of fungal species. The *Aspergillus* strains tested were isolated from patients treated at Memorial Sloan-Kettering Cancer Center from January 1985 to April 1987. The control organisms for amphotericin B were *Candida albicans* B311 and *C. tropicalis* CT4. The organisms (16 strains of *Aspergillus fumigatus*, 10 strains of *A. flavus*, and 10 strains of *A. niger*) were subcultured on Sabouraud dextrose agar and incubated at 35°C for 4 days. Spores were harvested with 0.02% Tween 80, centrifuged (10 min, 1,500 × *g*), suspended in distilled water, and counted in a hemacytometer. The spores were diluted in 2× yeast nitrogen glucose broth (Difco Laboratories; 5% glucose) to produce the high (10^5 spores per ml) and low (2×10^3 spores per ml) inocula.

Susceptibility testing. Portions of 0.1 ml of each spore suspension at either the high or low inoculum were added to microdilution tray wells that contained no drug (control wells), terbinafine, amphotericin B, or ketoconazole. Plates were incubated at 35°C for 48 h. The lowest concentration of test drug that prevented visible growth was considered the MIC. Wells without visible growth were subcultured on Sabouraud dextrose agar and incubated at 35°C for 48 h. Subcultures from wells with the lowest test drug concentration showing a 99.9% reduction from the initial inoculum size were judged to contain the minimal fungicidal concentration (MFC) of the test drug. Each organism was tested at least twice against each drug on at least 2 days.

With terbinafine and with amphotericin B, distinct endpoints were visible after 48 h of incubation (Table 1). The MICs of ketoconazole were >25.6 μg/ml for all strains. Results did not vary by more than one twofold dilution between different tests.

The MICs for the two *Candida* strains were >25.6 μg/ml for terbinafine, 0.4 μg/ml for amphotericin B, and <0.8 μg/ml for ketoconazole.

On the basis of the MICs for 90% of the strains (MIC_{90}s) and the MFCs for 90% of the strains (MFC_{90}s), amphotericin B was inhibitory or lethal against *A. fumigatus* at one-fourth the concentration of terbinafine. However, the MIC_{90} and MFC_{90} of terbinafine never exceeded 1.6 μg/ml. The MFCs of both drugs were close to the MICs, and inoculum size had no significant effect on terbinafine and amphotericin B results.

Terbinafine was active against *A. flavus* and *A. niger* at one-fourth the concentration of amphotericin B. Some strains were inhibited by concentrations as low as 0.025 μg/ml. The MFCs of both drugs were close to the MICs, and there was no significant inoculum effect.

Little information exists on the activity of terbinafine against aspergilli. In one in vitro study, the MIC_{90}s for *A. fumigatus* (11 strains) and *A. flavus* (9 strains) were 0.5 and 0.06 μg/ml, respectively (6). This study used Kimmig agar and an agar dilution method for in vitro testing. In a study using a broth dilution method with Sabouraud dextrose agar

* Corresponding author.

TABLE 1. Inhibitory and lethal activities of terbinafine and amphotericin B against *Aspergillus* species

Species (no. of strains)	Inoculum	Drug	MIC (μg/ml)			MFC (μg/ml)		
			Range	50%	90%	Range	50%	90%
A. fumigatus (16)	Low	Terbinafine	0.8–1.6	0.8	1.6	0.8–3.2	0.8	1.6
		Amphotericin B	0.2–0.4	0.4	0.4	0.2–0.4	0.4	0.4
	High	Terbinafine	0.8–1.6	1.6	1.6	0.8–3.2	1.6	1.6
		Amphotericin B	0.2–0.8	0.4	0.4	0.2–0.8	0.4	0.4
A. flavus (10)	Low	Terbinafine	0.025–0.4	0.2	0.4	0.025–0.4	0.4	0.4
		Amphotericin B	0.8–1.6	1.6	1.6	0.8–1.6	1.6	1.6
	High	Terbinafine	0.4–0.8	0.8	0.8	0.4–0.8	0.8	0.8
		Amphotericin B	0.8–3.2	1.6	3.2	0.8–3.2	1.6	3.2
A. niger (10)	Low	Terbinafine	0.025–0.4	0.1	0.4	0.05–0.4	0.1	0.4
		Amphotericin B	0.4–0.8	0.8	0.8	0.4–0.8	0.8	0.8
	High	Terbinafine	0.05–0.4	0.1	0.4	0.05–0.4	0.2	0.4
		Amphotericin B	0.4–1.6	0.8	1.6	0.4–1.6	0.8	1.6

and a low inoculum size (10^3 CFU/ml), the MICs for *A. fumigatus* ranged from 1.0 to 4.0 μg/ml, with a geometric mean MIC of 1.68 μg/ml (16 strains tested) (1). In another study with an unspecified number of strains, the calculated MIC for 50% of the strains (MIC_{50}) was 0.8 μg/ml (range, 0.05 to 1.56 μg/ml) (2). For *A. flavus*, the MICs ranged from 0.06 to 0.5 μg/ml, and the geometric mean MIC was 0.11 μg/ml (eight strains tested).

Our data show that terbinafine is highly active in vitro against *A. niger* and that it has activity superior to that of amphotericin B against *A. flavus* but somewhat inferior to that against *A. fumigatus*. The inoculum size did not significantly alter the susceptibility to terbinafine or amphotericin B of the aspergilli we tested. Ketoconazole did not show any inhibitory activity against the strains we tested.

The lethal activity of terbinafine has not been previously documented for *Aspergillus* species, and our results show that terbinafine is lethal against aspergilli at concentrations close to the MIC; again, the inoculum size did not have a major impact on the results.

Preliminary pharmacokinetic data for humans indicate that after a single oral dose of 500 mg of terbinafine, peak drug concentrations in plasma of 2 μg/ml are reached within 2 h and the elimination half-life is 11.3 h (8). These data, with the MIC_{90}s and MFC_{90}s below 2 μg/ml seen in our study, indicate that terbinafine is a promising new agent which should be tested in animal models of pulmonary aspergillosis.

This study was supported in part by a grant from Sandoz Ltd., Basel, Switzerland. H. J. Schmitt is supported by a grant from the Stiftung Volkswagenwerk, Hannover, Federal Republic of Germany.

LITERATURE CITED

1. **Clayton, Y. M.** 1987. The in vitro activity of terbinafine against uncommon fungal pathogens, p. 433–439. *In* R. A. Fromtling (ed.), Recent trends in the discovery, development and evaluation of antifungal agents. J. R. Prous Science Publishers, Barcelona, Spain.
2. **Petranyi, G., J. G. Meingassner, and H. Mieth.** 1987. Antifungal activity of the allylamine derivative terbinafine in vitro. Antimicrob. Agents Chemother. **31**:1365–1368.
3. **Ryder, N. S.** 1985. Specific inhibition of fungal sterol biosynthesis by SF 86-327, a new allylamine antimycotic agent. Antimicrob. Agents Chemother. **27**:252–256.
4. **Ryder, N. S., and M. C. Dupont.** 1985. Inhibition of squalene epoxidase by allylamine antimycotic compounds. A comparative study of the fungal and mammalian enzymes. Biochem. J. **230**: 765–770.
5. **Ryder, N. S., G. Seidl, and P. F. Troke.** 1984. Effect of the antimycotic drug naftifine on growth of and sterol biosynthesis in *Candida albicans*. Antimicrob. Agents Chemother. **25**:483–487.
6. **Shadomy, S., A. Espinel-Ingroff, and R. J. Gebhart.** 1985. In vitro studies with SF 86-327, a new orally active allylamine derivative. Sabouraudia **23**:125–132.
7. **Stephan, A., R. Czok, and O. Maly.** 1987. Terbinafine—initial clinical results, p. 511–520. *In* R. A. Fromtling (ed.), Recent trends in the discovery, development and evaluation of antifungal agents. J. R. Prous Science Publishers, Barcelona, Spain.
8. **Stutz, A., and G. Petranyi.** 1984. Synthesis and antifungal activity of (E)-*N*-(6,6-dimethyl-2-hepten-4-ynyl)-*N*-methyl-1-naphthalene-methanamine (SF 86-327) and related allylamine derivatives with enhanced oral activity. J. Med. Chem. **27**:1539–1543.