JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2004, p. 4319–4320 Vol. 42, No. 9
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.9.4319–4320.2004

In Vitro Susceptibilities of Isolates of *Sporothrix schenckii* to Itraconazole and Terbinafine

Lidiane Meire Kohler,[1] Paulo César Fialho Monteiro,[2] Rosane Christine Hahn,[1] and Júnia Soares Hamdan[1]*

Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais,[1] and Instituto de Pesquisa Clínica Evandro Chagas, Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro,[2] Brazil

Received 10 February 2004/Returned for modification 29 March 2004/Accepted 16 May 2004

Thirty isolates of the yeast form of *Sporothrix schenckii* were evaluated for in vitro susceptibility to itraconazole and terbinafine by the recommended NCCLS modified technique (M27-A2). The MICs of itraconazole obtained oscillated between 0.062 and 4.0 μg/ml, and those of terbinafine oscillated between 0.007 and 0.50 μg/ml; therefore, terbinafine showed greater in vitro activity.

Sporotrichosis is a subacute or chronic infection affecting both animals and humans and is characterized by nodular cutaneous and subcutaneous lesions, which may involve the adjacent lymphatic system, which suppurates and drains (1, 20).

Itraconazole is currently considered the treatment of choice to treat the diverse clinical manifestations of sporotrichosis (13, 14, 17, 18). On the other hand terbinafine by virtue of its excellent in vitro and in vivo activity is under comparative evaluation for its therapeutic potential for a wide range of fungal infections (4, 6, 8, 11, 21, 22).

Promising in vitro results with terbinafine for both the fixed and the lymphocutaneous forms of sporotrichosis due to the fungus *Sporothrix schenckii* (10, 11, 21) are being compared and confirmed clinically (4, 8, 10, 19).

In this study, our objective was to determine the in vitro efficacy of terbinafine against isolates of *Sporothrix schenckii* by the technique of macrodilution in a liquid medium (NCCLS M27-A2) (15) adapted for dimorphic fungi.

Thirty strains of *Sporothrix schenckii*, including 2 reference isolates (ATCC 201679 and M527-88), 18 human clinical isolates, and 10 animal isolates (9 from cats and 1 from a horse) were included in this study. All the samples were isolated from clinical specimens, identified by micromorphological characteristics and demonstration of typical dimorphism. They were maintained in brain heart infusion (BHI) solid medium–0.5% glucose at a controlled temperature of 4°C, with replication onto new medium at 6-month intervals, and converted to the yeast form through successive passages in BHI solid medium and incubation at 35°C. Quality control (QC) strains *Candida krusei* (ATCC 6258) and *Candida parapsilosis* (ATCC 22019) were tested in parallel and were inhibited by MICs at the correct range for the antifungal tested (itraconazole) (15). The MIC range of terbinafine for the QC *Candida* strains has not yet been established by the NCCLS.

Susceptibility tests were conducted using a technique of macrodilution in a liquid medium in accordance with the NCCLS protocol (M27-A2) (15), adapted for dimorphic fungi to include a 5-day incubation period to compensate for the sluggish growth of the yeast phase of *Sporothrix schenckii*, which requires 5 days to reach exponential growth (3, 7) and the addition of glucose to the medium (20 g/liter). The inoculum was prepared spectrophotometrically (520 nm, 60% of transmittance) to reach approximately 1×10^6 to 5×10^6 CFU/ml, at an incubation temperature of 35°C.

The drugs itraconazole (Janssen Pharmaceutical, Beerse, Belgium) and terbinafine (Novartis Research Institute, Vienna, Austria) were obtained in their pure form, dissolved in dimethyl sulfoxide, and prepared at a 10× concentration in RPMI 1640 (GIBCO).

Before being added to test tubes the suspensions were diluted to 1:100 and 1:20 in the RPMI 1640-2% glucose to reach a final concentration of 0.5×10^3 to 2.5×10^3 CFU/ml. Samples of 0.1 ml of the drugs at each concentration were transferred to 12- by 75-mm test tubes, in duplicate, to which were added 0.9 ml of the inoculum previously prepared in a spectrophotometer (520 nm, 60% T). The samples of diluted drugs corresponded to a range of 0.007 to 4.0 μg/ml. All assays were performed in triplicate.

All tubes were incubated at 35°C under constant agitation in a thermoshaker (Gerhardt, Königswinter, Germay) for 5 days. The MICs were read and checked visually for inhibition of fungal growth by approximately 80% in relation to growth in control tubes, where 100% of growth was visualized.

The MICs of itraconazole obtained for the human clinical isolates oscillated between 0.062 and 4.0 μg/ml, and those of terbinafine oscillated between 0.007 and 0.50 μg/ml. With respect to the animal isolates, itraconazole yielded consistent results of 4.0 μg/ml for all the cat samples and a value of 0.062 μg/ml for the horse isolate and terbinafine yielded values between 0.007 and 0.125 μg/ml for both cat and horse isolates (Table 1).

For itraconazole the MICs reported here are in agreement with the literature, which reports low levels of resistance for cells of *Sporothrix schenckii* to this antifungal drug (3, 5, 9, 14). It should be emphasized that the values obtained for the iso-

* Corresponding author. Mailing address: Departamento de Microbiologia, ICB/ UFMG, Av. Antônio Carlos, 6627, 31270-901 Belo Horizonte, Minas Gerais, Brazil. Phone: 55 31 3499-2758. Fax: 55 31 3499-2730. E-mail: handan@icb.ufmg.br.

TABLE 1. Susceptibilities of 30 isolates of *Sporothrix schenckii* to itraconazole and terbinafine, including QC strains of *Candida*

Species and group of isolates (*n*) or strain	Antifungal agent	MIC (μg/ml)[a]			% Resistant isolates
		Range	50%	90%	
S. schenckii					
Animal (10)	Itraconazole	0.062–4.0[b]	4.0	4.0	90
	Terbinafine	0.007–0.125	0.062	0.125	0
Human (20)	Itraconazole	0.031–4.0	0.062	4.0	25
	Terbinafine	0.007–0.50	0.031	0.25	0
C. krusei ATCC 6258 (QC)	Itraconazole	0.125–0.50	0.25	0.50	0
	Terbinafine	4.0–>4.0	>4.0	>4.0	100
C. parapsilosis ATCC 22019 (QC)	Itraconazole	0.125–0.25	0.25	0.25	0
	Terbinafine	0.25–0.50	0.25	0.50	0

[a] 50% and 90%, MICs at which 50 and 90% of isolates, respectively, are inhibited.
[b] Only one susceptible isolate, from a horse.

lates from cats indicated resistance to itraconazole. This was attributed to the high frequency of serious forms with systemic dissemination of sporotrichosis among cats, and, because of the scarce data available on the use of azole derivatives in the treatment of animal sporotrichosis, no comparison of the data obtained here was possible (2, 16).

In relation to terbinafine no references in the current literature to in vitro activity against the yeast form of *Sporothrix schenckii* were found. However, some references made mention of the mycelium phase (11, 21).

In the present study, we are able to report the extreme susceptibility of all human and animal isolates of *Sporothrix schenckii* to terbinafine. The data reveal its potent in vitro activity against the yeast cells of *Sporothrix schenckii*, which is in agreement with the available literature, although with reference to other fungi. The potential in vivo therapeutic value of terbinafine has been confirmed, up to the present, only for cases of cutaneous and lymphocutaneous sporotrichosis (8, 11, 19, 21), although the use of elevated doses of the drug have been suggested (above 500 mg day^{-1}) to ensure clinical efficacy (4). Efficacy has not been observed with the use of terbinafine for the treatment of systemic sporotrichosis in the murine model (12). There is an obvious need for further studies to correlate the in vitro susceptibility tests with the clinical response of patients with various clinical forms of sporotrichosis.

We thank Novartis Research Institute (terbinafine) and Janssen Pharmaceutical (itraconazole) for providing the drugs in their pure form. We also thank Jacqueline Travassos de Mello and Mariângela Ferreira Verardo (Hospital Universitário de Juiz de Fora, Minas Gerais, Brazil) for providing 10 samples used in this study.

This research was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Conselho Nacional de Desenvolvimento Científico e Tecnológico, and Fundação de Amparo à Pesquisa do Estado de Minas Gerais.

REFERENCES

1. **Araújo, T., A. C. Marques, and F. Kerdel.** 2001. Sporotrichosis. Int. J. Dermatol. **40:**737–742.
2. **Barros, M. B. L., T. M. P. Schubach, M. C. G. Galhardo, A. O. Schubach, P. C. F. Monteiro, R. S. Reis, R. M. Z. Oliveira, M. S. Lazera, T. C. Maya, T. C. M. Blanco, K. B. F. Marzochi, B. Wanke, and A. C. F. Valle.** 2001. Sporotrichosis: an emergent zoonosis in Rio de Janeiro. Mem. Inst. Oswaldo Cruz **96:**777–779.
3. **Casali, A. K., and J. S. Hamdan.** 1997. Effect of three azole derivatives on the lipids of different strains of *Sporothrix schenckii.* Can. J. Microbiol. **43:**1197–1202.
4. **Chapman, S. W., P. Pappas, C. Kauffman, E. B. Smith, R. Dietze, N. Tiraboschi-Foss, A. Restrepo, A. B. Bustamante, C. Opper, S. Emady-Azar, and R. Bakshi.** 2004. Comparative evaluation of the efficacy and safety of two doses of terbinafine (500 and 1000 mg day^{-1}) in the treatment of cutaneous or lymphocutaneous sporotrichosis. Mycoses **47:**62–68.
5. **Espinel-Infroff, A.** 1998. In vitro activity of the new triazole voriconazole (UK-109,496) against opportunistic filamentous and dimorphic fungi and common and emerging yeast pathogens. J. Clin. Microbiol. **36:**198–202.
6. **Hahn, R. C., C. J. F. Fontes, R. D. Batista, and J. S. Hamdan.** 2002. In vitro comparison of activities of terbinafine and itraconazole against *Paracoccidioides brasiliensis*. J. Clin. Microbiol. **40:**2828–2831.
7. **Hamdan, J. S., and A. K. Casali.** 1996. Effect of amphotericin B on the lipids of yeast cells of *Sporothrix schenckii.* Mycopathologia **136:**125–131.
8. **Hay, R. J.** 1999. Therapeutic potential of terbinafine in subcutaneous and systemic mycoses. Br. J. Dermatol. **141**(Suppl. 56)**:**36–40.
9. **Heeres, J., L. J. J. Backx, and J. Van Cutsem.** 1984. Actimomycotic azoles: synthesis and antimycotic properties of R-51211 and its congeners. J. Med. Chemother. **27:**894–900.
10. **Hull, P. R., and H. F. Vismer.** 1992. Treatment of cutaneous sporotrichosis with terbinafine. Br. J. Dermatol. **126**(Suppl. 39)**:**51–55.
11. **Jessup, C. J., N. S. Ryder, and M. A. Ghannoum.** 2000. An evaluation of the *in vitro* activity of terbinafine. Med. Mycol. **38:**155–159.
12. **Kan, V. L., and J. E. Bennett.** 1988. Efficacies of four antifungal agents in experimental murine sporotrichosis. Antimicrob. Agents Chemother. **32:** 1619–1623.
13. **Kauffman, C. A., R. Hajjeh, and S. W. Chapman.** 2000. Practice guidelines for the management of patients with sporotrichosis. Clin. Infect. Dis. **30:** 684–687.
14. **Koç, A. N., U. Uksal, and O. Oymak.** 2001. Case report. Successfully treated subcutaneous infection with *Sporothrix schenckii* in Turkey. Mycoses **43:**75–77.
15. **National Committee for Clinical Laboratory Standards.** 2002. Reference method for broth dilution antifungal susceptibility testing of yeast. Approved standard M27–A2. National Committee for Clinical Laboratory Standards, Wayne, Pa.
16. **Nobre, M. O., A. P. Castro, D. Caetano, L. L. Souza, M. C. A. Meireles, and L. Ferreiro.** 2001. Recurrence of sporotrichosis in cats with zoonotic involvement. Rev. Iberoam. Micol. **18:**137–140.
17. **Noguchi, H., M. Hiruma, and A. Kawada.** 1999. Case report. Sporotrichosis successfully treated with itraconazole in Japan. Mycoses **42:**571–576.
18. **Peña, C., J. Garcia-Silva, J. Varela, M. Ardavín, and M. Pereiro, Jr.** 1999. Esporotricosis cutánea atípica. Respuesta a itraconazol y cirugía. Acta Otorrinolaringol. Esp. **50:**485–489.
19. **Pérez, A.** 1999. Terbinafine: broad new spectrum of indications in several subcutaneous and systemic and parasitic diseases. Mycoses **42**(Suppl. 2)**:**111–114.
20. **Rippon, J. W.** 1988. Sporotrichosis, p. 277–302. *In* J. W. Rippon (ed.), Medical mycology. The pathogenic fungi and the pathogenic actinomycetes, 3rd ed. The W. B. Saunders Company, Philadelphia, Pa.
21. **Ryder, N. S.** 1999. Activity of terbinafine against serious fungal pathogens. Mycoses **42**(Suppl. 2)**:**115–119.
22. **Tanuma, H., M. Hiramatsu, H. Mukai, M. Ab, H. Kume, S. Nishiyama, and K. Katsuoka.** 2000. Case report: a case of chromoblastomycosis effectively treated with terbinafine. Characteristics of chromoblastomycosis in the Kitasato region, Japan. Mycoses **43:**79–83.