In Vitro Activities of Four Novel Triazoles against *Scedosporium* spp.

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In order to develop new approaches to the treatment of the severe and usually fatal infections caused by *Scedosporium* spp., the in vitro antifungal activities of four novel triazoles (posaconazole, ravuconazole, voriconazole, and UR-9825) and some current antifungals (amphotericin B, ketoconazole, itraconazole, and nystatin) were determined. The latter group was clearly ineffective against the two species tested. The four new antifungals showed activity against *Scedosporium apiospermum*, and UR-9825 and voriconazole were active against *S. prolificans*.

The anamorph genus *Scedosporium* comprises two important pathogens to humans, *Scedosporium apiospermum*, the asexual state of *Pseudallescheria boydii*, and *S. prolificans*. Previously, both species were associated mainly with localized infections. Generally, *S. apiospermum* was associated with white-grain mycetoma and *S. prolificans* was associated with subcutaneous infections, with a predilection for cartilage and joint areas (2, 4, 6, 14). However, in recent years both species have evolved into important agents causing disseminated infections, mainly in neutropenic patients. Unfortunately, these infections are usually unresponsive to antifungals and their management becomes very difficult.

In disseminated-infection cases, *S. apiospermum* shows a marked predilection for the central nervous system (9). The invasive infections caused by *S. prolificans* usually involve patients with underlying blood malignancies and are usually fatal. These patients have generally been treated with amphotericin B and occasionally with other antifungals, such as itraconazole, flucytosine, fluconazole, or miconazole, but commonly with unsuccessful outcomes. The prognosis is worse for *S. prolificans* infections because in only a few cases has the infection been overcome and then only after resolution of the underlying neutropenia. The in vitro susceptibility of *S. prolificans* to the traditional antifungals has been tested in several studies (3, 8, 15; J. Guarro, J. Cano, J. Gené, M. Solé, and A. J. Carrillo-Muñoz, Abstr. 14th Congr. Int. Soc. Hum. Anim. Mycol., p. 84; P. Pacheco, D. Sutton, A. Fothergill, and M. Rinaldi, Abstr. 14th Congr. Int. Soc. Hum. Anim. Mycol., abstr. 79, p. 234), and although the methodological conditions differed between the laboratories, in general, their results correlate with the observed poor clinical responses. In this study, we evaluated the inhibitory activities of four novel triazoles (ravuconazole, posaconazole, voriconazole, and UR-9825) and some current ones, such as amphotericin B, ketoconazole, itraconazole, and nystatin, against 33 clinical isolates of *S. prolificans* and 11 of *S.*

apiospermum. The isolates were tested by a previously described microdilution method (12), using sterile, 96-well microplates, mainly according to the guidelines of the National Committee for Clinical Laboratory Standards for molds (11).

The isolates, from very diverse clinical sources, were supplied by different Spanish hospitals. They were identified at the Microbiology Unit of the Medical School of the Rovira i Virgili University at Reus, Spain. The isolates, stored in sterile, distilled water until the time of study, were subcultured onto potato dextrose agar (Difco Laboratories, Detroit, Mich.) at 30°C for 7 days to ensure the viability, purity, and sporulation of the inoculum. Amphotericin B, ravuconazole, nystatin (Bristol-Myers Squibb, Princeton, N.J.), ketoconazole, itraconazole (Janssen Research Foundation, Beerse, Belgium), voriconazole (Pfizer Ltd., Sandwich, United Kingdom), posaconazole (Schering-Plough, Kenilworth, N.J.), and UR 9825 (J. Uriach & Cia, SA, Barcelona, Spain) were tested in RPMI 1640 medium buffered to pH 7 with 0.165 M morpholinepropanesulfonic acid (MOPS). Aliquots of 100 µl of the drug dilutions were inoculated into the wells of microplates, which were stored at –70°C until they were used. The inoculum was prepared by removing the sporulated fungi from the agar slant with a loop and suspending them in 10 ml of sterile water. The suspension was adjusted to 68 to 70% transmittance at 530 nm and diluted 10-fold to yield a working suspension of 0.4×10^4 to 5×10^4 CFU/ml. The concentrations of the test drugs were 0.016 to 16 µg/ml. The temperature of incubation was 35°C, and MIC readings were made after 48 and 72 h. The amphotericin B and nystatin MICs were defined as the lowest drug concentrations at which there was a complete absence of growth. The MICs of the azoles were defined as the lowest drug concentrations that led to a 50% inhibition of growth in comparison to controls. *Paecilomyces variotii* ATCC 36257 was used as a quality control strain. The data are presented as MIC ranges, geometric mean MICs, and the MICs required to inhibit 50 and 90% of the isolates of each species (MIC₅₀ and MIC₉₀, respectively).

All isolates produced detectable growth after 72 h of incubation; therefore, MICs were determined at this time interval. The MICs of all eight drugs for all 44 isolates are summarized

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TABLE 1. Antifungal susceptibilities of *Scedosporium* spp.^a

Fungus (no. of isolates tested) and drug	MIC range	Geometric mean MIC	MIC ₅₀	MIC ₉₀
<i>S. prolificans</i> (33)				
Amphotericin B	0.125–>16	6.48	8	16
Nystatin	4–>16	4.13	>16	>16
Itraconazole	8–>16	15.36	>16	>16
Ketoconazole	0.5–16	11.52	>16	>16
Ravuconazole	0.5–>16	8.9	16	16
Posaconazole	0.25–>16	10.55	16	16
Voriconazole	0.06–4	1.83	2	4
UR-9825	0.01–4	0.35	0.5	2
<i>S. apiospermum</i> (11)				
Amphotericin B	2–>16	4	4	>16
Nystatin	8–>16	13.24	16	>16
Itraconazole	0.03–>16	4.5	>16	>16
Ketoconazole	4–16	10.07	16	>16
Ravuconazole	0.125	0.125	0.125	0.125
Posaconazole	0.03–0.25	0.08	0.03	0.25
Voriconazole	0.01–0.25	0.06	0.125	0.25
UR-9825	1	1	1	1

^a All MICs are in micrograms per milliliter.

in Table 1. The four current antifungals (amphotericin B, nystatin, itraconazole, and ketoconazole) showed very high MICs against both species. Amphotericin B, the drug commonly used to treat these infections, presented geometric mean MICs of 6.48 µg/ml against *S. prolificans* and 4 µg/ml against *S. apiospermum*. We observed a strain-dependent in vitro response to amphotericin B, as pointed out by Walsh et al. (15); thus, while the MICs for most of the strains were high, two strains of *S. prolificans* were susceptible to this drug (MIC, 0.125 µg/ml). By contrast, for all of the strains of *S. apiospermum*, the MICs were ≥2 µg/ml. The results displayed by the four novel triazoles varied according to the species. Ravuconazole, posaconazole, and voriconazole were very active against *S. apiospermum*, their geometric mean MICs being 0.125, 0.08, and 0.06 µg/ml, respectively, and none showed a MIC higher than 0.25 µg/ml against any strain. UR-9825 showed slightly lower activity against this species, i.e., a mean MIC of 1 µg/ml. In the case of *S. prolificans*, the results obtained with the four triazoles were the reverse of those against the other species; i.e., UR-9825 showed the best activity. The mean MICs of ravuconazole, posaconazole, and voriconazole were 8.9, 10.5, and 1.83 µg/ml, respectively, and that of UR-9825 was 0.35 µg/ml. The MICs for the quality control strain were as follows: amphotericin B, 0.06 µg/ml; nystatin, 0.125 µg/ml; itraconazole and ketoconazole, 0.03 µg/ml; ravuconazole, posaconazole, voriconazole, and UR-9825, 0.06 µg/ml.

The results obtained with amphotericin B, itraconazole, and ketoconazole agree with those of other authors who used the same microdilution method and followed the NCCLS guidelines (11). However, in the case of voriconazole, we observed important discrepancies. Cuenca-Estrella et al. (3) reported a MIC range of 8 to 32 µg/ml and a MIC₉₀ of 16 µg/ml against *S. prolificans* and a MIC range of 0.5 to 2 µg/ml and a MIC₉₀ of 2 µg/ml against *S. apiospermum*. In our study, these values were considerably lower, i.e., 0.06 to 4 and 4 µg/ml, respectively, against *S. prolificans* and 0.01 to 0.25 and 0.25 µg/ml, respectively, against *S. apiospermum*. These discrepancies can

be explained, in part, by some differences in methodological procedure. Those authors used RPMI medium–2% glucose and a final inoculum suspension of 10⁵ CFU/ml, and the MIC was always defined as the lowest concentration that completely inhibited fungal growth. We used the 50% reduction endpoint. If, instead, we had used the criterion of complete inhibition, the MICs would have increased, in general, by more than 2 dilutions. Despite its being generally accepted that the MIC endpoint definition is crucial, there is still no universal consensus on what is appropriate. However, for voriconazole, some clinical results exist on the treatment of *S. apiospermum* infections that seem to correlate better with lower MICs, i.e., those obtained with the less strict criterion. Treatment with voriconazole has had a good clinical outcome for three patients with *S. apiospermum* invasive infection (5, 7, 10). Therefore, the MICs obtained in our study are apparently more predictive than those obtained by Cuenca-Estrella et al. (3). With *S. apiospermum*, we also observed important differences from the results of other authors. Walsh et al. (15) reported mean MICs of amphotericin B and itraconazole of 1.1 and 0.45 µg/ml, respectively, versus the 4 and 4.5 µg/ml obtained in our study. Those authors used the broth macrodilution method, and although we have demonstrated here that the broth macro- and microdilution methods for testing of the antifungal susceptibilities of some filamentous fungi (12) produced similar results, the use of two different techniques could be the cause of such differences.

It is worth mentioning that in our study, UR-9825 was very effective against *S. prolificans*. This drug is a potent new triazole derivative that showed efficacy in vitro against pathogenic yeasts (13) and in animal models of candidiasis and aspergillosis (1), and our results suggest that UR-9825 should be considered for further study in the treatment of *S. prolificans*. Another promising approach is the combination of antifungal drugs with different mechanisms of action. Meletiadis et al. (8) have recently reported an in vitro synergistic effect of the terbinafine-itraconazole combination against *S. prolificans*. Walsh et al. (15) reported a lack of in vitro fungicidal effect of either amphotericin B or azoles against *S. apiospermum*. By contrast, they noticed augmented antifungal activity with amphotericin B-azole (miconazole, itraconazole, or fluconazole) combinations. Further studies with these approaches in appropriate animal models are required in order to develop therapeutic strategies for the treatment of the severe and nearly always fatal infections caused by these fungi in neutropenic patients.

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