

Comparative in-vitro activity of voriconazole (UK-109,496) and six other antifungal agents against clinical isolates of *Scedosporium prolificans* and *Scedosporium apiospermum*

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We report the in-vitro susceptibility of 27 clinical isolates of *Scedosporium apiospermum* and 43 of *Scedosporium prolificans*. *S. apiospermum* was resistant to fluconazole and flucytosine, with variable susceptibility to amphotericin B, itraconazole, ketoconazole and susceptible to miconazole. Voriconazole was much more active than fluconazole and flucytosine, more active than amphotericin B, itraconazole and ketoconazole and was as active as miconazole against *S. apiospermum* isolates. Voriconazole and the other six antifungal agents showed low activity against *S. prolificans* isolates.

Introduction

Scedosporium apiospermum (*Pseudallescheria boydii*) and *Scedosporium prolificans* are members of the dematiaceous hyphomycetous genus *Scedosporium*.¹ These moulds are pathogenic in humans, causing asymptomatic colonization, localized infections following penetrating trauma or intravenous drug abuse, and disseminated infections particularly in immunosuppressed patients.¹ They are widespread in nature as soil saprophytes.²

Optimal treatment of these fungal infections is unknown. Debridement or excision of necrotic tissue and antifungal chemotherapy should be the treatment of choice,³ but indications for surgery are limited, and dose and duration of chemotherapy have not been established. Results of treatment have been variable. Prognosis depends mainly on the patient's immune status and feasibility of surgical debridement.³ In neutropenic patients with disseminated infection, death is the usual outcome despite antifungal treatment.^{1,3}

Little is known about the susceptibility of *Scedosporium* spp. to antifungal agent.^{3,4} Isolates of *S. apiospermum* are considered to be susceptible to miconazole and resistant to fluconazole and flucytosine; they appear to have variable susceptibility to itraconazole, ketoconazole and amphotericin B.⁴ *S. prolificans* is multi-resistant: recent studies

show that antifungal drugs have low activity against this organism *in vitro*.³

Voriconazole (UK-109,496) is a new, broad-spectrum triazole with activity against *Candida* spp., *Cryptococcus neoformans* and some moulds.⁴ Animal studies and recently published clinical data suggest that it is effective in the treatment of invasive mycoses.⁵ In the present study we describe the results of in-vitro determinations of susceptibility to voriconazole and other antifungal agents carried out on 27 clinical isolates of *S. apiospermum* and 43 of *S. prolificans*.

Materials and methods

All mould isolates tested were obtained from clinical specimens, from more than 20 different Spanish hospitals between 1992 and March 1998. Their sites of isolation are listed in Table I. Susceptibility testing was performing according to the NCCLS reference micromethod, with minor modifications.⁶ The medium used for susceptibility testing was RPMI 1640 with L-glutamine (Sigma-Aldrich Química, Madrid, Spain) buffered with morpholine propanesulphonic acid (MOPS) (Sigma-Aldrich Química) to a final molarity of 0.165 M, adjusted to pH 7.0 by using 10 M NaOH, and supplemented with 18 g/L of glucose

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Table I. Sites of isolation of *Scedosporium* spp. obtained from clinical samples

Sources	<i>S. apiospermum</i> (n = 27)	<i>S. prolificans</i> (n = 43)
Blood cultures	–	18
Skin biopsy	4	3
Sputum	7	4
Bronchoalveolar lavage	2	4
Synovial fluid	3	2
Bronchial aspirate	2	3
Corneal scraping	2	–
Paranasal sinus biopsy	–	2
Cerebrospinal fluid	1	1
Faeces	–	1
Ear exudate	1	1
Surgical drainage fluid	1	–
Vascular biopsy	1	–
Intraocular lens	1	–
Unspecified	2	4

(RPMI-2% glucose).⁷ The antifungal drugs used in the susceptibility testing procedure were amphotericin B (Squibb, Madrid, Spain), flucytosine (Roche, Madrid, Spain), ketoconazole, miconazole and itraconazole (Jansen Pharmaceutica, Madrid, Spain), and fluconazole and voriconazole (UK-109,496; Pfizer Ltd, Sandwich, UK). All seven initial solutions of antifungal agents were diluted with RPMI-2% glucose. The concentration of voriconazole ranged from 64 to 0.125 mg/L. The concentrations of the other six antifungal agents ranged from 16 to 0.03 mg/L. The microtitre plates contained two-fold serial dilutions of the antifungal drugs and two drug-free medium wells for sterility and growth controls.

Inoculum suspensions were standardized to get a spectrophotometric reading of 68–71% transmission at 530 nm. Thus, final suspensions containing $1-5 \times 10^6$ cfu/mL were obtained. Each well contained 10 μ L of inoculum suspension (*c.* 10^5 cfu/mL). The microtitre plates were incubated at 35°C in humid atmosphere for 48 h and read macroscopically with a mirror. The MIC was defined as the lowest concentration of the antifungal agent that completely inhibited fungal growth.

Paecilomyces variotii (ATCC 22319), *Aspergillus fumigatus* (ATCC 9197) and *Candida parapsilosis* (ATCC 22019) were used as reference strains to control the quality and to monitor the reproducibility of susceptibility tests. The MIC values of 30 consecutive determinations varied by no more than two two-fold dilutions. The MICs of voriconazole were 0.06–0.25 mg/L for *P. variotii*, 0.5–1 mg/L for *A. fumigatus* and 0.007–0.03 mg/L for *C. parapsilosis*.

Results

The in-vitro susceptibility results are summarized in Table II. *S. apiospermum* had variable susceptibility to the seven antifungal drugs tested. Six isolates were inhibited by 2 mg/L of amphotericin B, and seven by 4 mg/L. For all *S. apiospermum* isolates the flucytosine and fluconazole MICs were very high. However, four isolates of *S. apiospermum* were inhibited by itraconazole concentrations ≤ 2 mg/L, and six by 4 mg/L. Thirteen (50%) of the *S. apiospermum* isolates were inhibited by ketoconazole concentrations of ≤ 2 mg/L. The in-vitro activity of miconazole against *S. apiospermum* seemed better than that of the other antifungal drugs tested; 17 isolates were inhibited by miconazole concentrations of ≤ 1 mg/L. Voriconazole MICs were ≤ 1 mg/L for 22/27 isolates. In all cases voriconazole was more potent than flucytosine, amphotericin B, fluconazole, itraconazole and ketoconazole. In contrast to *S. apiospermum*, the in-vitro activity of all antifungal drugs tested was very low against *S. prolificans* (Table II).

Discussion

In order to ascertain the susceptibility of *Scedosporium* spp. we studied the in-vitro activity of seven antifungal agents against 27 clinical isolates of *S. apiospermum* and 43 of *S. prolificans*.

Miconazole appears to be the antifungal agent of choice to treat *S. apiospermum* infections, but it is associated with several adverse effects.³ Itraconazole is a good alternative in some cases, but is poorly absorbed after oral administration, although a new oral formulation is absorbed much more reliably.^{8,9} *S. apiospermum* infections are difficult to treat: fluconazole and flucytosine lack activity against this organism.^{3,4}

New therapeutic approaches are clearly needed for treatment of these mycoses. In our study, *S. apiospermum* isolates seem to be susceptible to voriconazole (most MICs were ≤ 1 mg/L). Voriconazole was much more active than fluconazole and flucytosine, more active than amphotericin B, itraconazole and ketoconazole and showed good activity (comparable to that of miconazole) against *S. apiospermum* isolates. In patients receiving voriconazole 200 mg po bd for the treatment of acute and chronic invasive aspergillosis, the concentration of this antifungal agent in serum was 3.27 mg/L; this bioavailability warrants clinical studies.¹⁰

Our in-vitro susceptibility results show that *S. prolificans* is multi-resistant. Voriconazole, like itraconazole, had low activity against *S. prolificans*, this activity was slightly higher than that of the other antifungal drugs studied.

Few data correlating susceptibilities to in-vitro azole agents with response to therapy *in vivo* are available,

Table II. In-vitro susceptibility of *Scedosporium* spp. to voriconazole and other antifungal agents

Species	Antifungal agent	MIC (mg/L)			
		range	mode	MIC ₅₀	MIC ₉₀
<i>S. prolificans</i> (n = 43)	amphotericin B	8->16	>16	>16	>16
	flucytosine	>16	>16	>16	>16
	fluconazole	>16	>16	>16	>16
	itraconazole	8->16	16	16	>16
	ketoconazole	>16	>16	>16	>16
	miconazole	>16	>16	>16	>16
	voriconazole	8-32	16	16	16
<i>S. apiospermum</i> (n = 27)	amphotericin B	2->16	4	4	16
	flucytosine	>16	>16	>16	>16
	fluconazole	16->16	>16	>16	>16
	itraconazole	2-16	8	8	16
	ketoconazole	0.06->16	2	2	>16
	miconazole	0.06-4	0.5	0.5	2
	voriconazole	0.5-2	1	1	2

especially from patients with mould infection. However, the low MICs and high bioavailability of voriconazole are promising and indicate that this new triazole should be a suitable agent, even in the immunocompromised host, for the treatment of *S. apiospermum* infections. The in-vitro susceptibility results of voriconazole suggest that the drug may be ineffective against *S. prolificans*.

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