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UK BACTERIAL ENDOCARDITIS WORKSHOPS

October/November 1998

The British Society for Antimicrobial Chemotherapy (BSAC), in collaboration with the Association of Medical Microbiologists (AMM) and the Hospital Infection Society (HIS), is holding a series of educational workshops focusing on bacterial endocarditis.

Dulwich – Monday 19 October
Darlington – Wednesday 21 October
Chester – Thursday 22 October
Bristol – Friday 23 October
Derby – Wednesday 28 October
Peterborough – Thursday 5 November
Reading – Tuesday 10 November
Dublin – Date to be advised
Stirling – Date to be advised

Registration Fee: £25.00 (BSAC/AMM/HIS members), £100.00 (non-members)

The meetings will commence with a buffet lunch at 12 noon. The workshops will begin at 1.00pm and will close at approximately 4.30pm.

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Voriconazole against fluconazole-susceptible and resistant candida isolates: in-vitro efficacy compared with that of itraconazole and ketoconazole

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We compared the in-vitro activity of fluconazole, itraconazole, ketoconazole and voriconazole against 67 blood isolates of *Candida* spp. exhibiting a wide range of fluconazole MICs (0.125 to >64 mg/L). Voriconazole was the most potent *in vitro*, followed by itraconazole, ketoconazole and fluconazole. Itraconazole and voriconazole had in-vitro activity against fluconazole-susceptible and -resistant candida isolates. Higher itraconazole and voriconazole MICs were observed in isolates exhibiting higher fluconazole MICs, suggesting cross-resistance. Itraconazole and voriconazole MICs of ≥16 mg/L were observed only in *Candida albicans* and *Candida tropicalis*. *Candida krusei* and *Candida glabrata* exhibited itraconazole MICs of 0.5–1 mg/L and voriconazole MICs of 0.25–0.5 mg/L.

Introduction

Voriconazole is a new triazole antifungal agent which acts by inhibiting cytochrome P450 sterol 14α-demethylase, an enzyme involved in ergosterol biosynthesis. Voriconazole has potent in-vitro and in-vivo activity against *Aspergillus* spp. and other moulds.[1–3] Although voriconazole has in-vitro activity against fluconazole-resistant *Candida albicans*, *Candida krusei* and *Candida glabrata*,[4,5] its activity against other *Candida* spp. that are fluconazole-resistant *in vitro* is unknown. Furthermore, the in-vitro activity of voriconazole has not been compared with that of itraconazole and ketoconazole. The goal of this study was to compare the in-vitro activity of fluconazole, itraconazole, ketoconazole and voriconazole against a large number of candida isolates; the isolates studied exhibited a wide range of fluconazole MICs.

Materials and methods

Sixty-seven blood isolates of *Candida* spp. collected during a prospective study of candidaemia were tested.[6] These isolates exhibited fluconazole MICs ranging from 0.125 to >64 mg/L. These included *C. albicans* (24 isolates), *Candida tropicalis* (17), *C. glabrata* (12), *Candida parapsilosis* (8), *C. krusei* (3) and *Candida lusitaniae* (3). *C. parapsilosis* ATCC 90018, *C. albicans* ATCC 90028 and 90029 and *C. glabrata* ATCC 90030 were incorporated into each set of experiments as quality control isolates.

The susceptibility testing was performed by a macrodilution method adhering to the National Committee for Clinical Laboratory Standards (NCCLS) protocol.[7] Fluconazole (Pfizer Central Research, Groton, CN, USA) stock solutions of 2000 mg/L were prepared with sterile distilled water. Voriconazole (Pfizer Central Research, Groton, CN, USA) stock solutions of 4000 mg/L were prepared with dimethylsulphoxide (DMSO); subsequent dilutions were performed in water. Stock solutions of ketoconazole and itraconazole (Janssen Research Foundation, Beerse, Belgium) were prepared with 0.2 N HCl and DMSO, respectively; subsequent drug dilutions were performed according to the manufacturer's protocol. The concentrations of drugs tested were: 0.125–64 mg/L for fluconazole; 0.015–16 mg/L for itraconazole and voriconazole; and 0.03–16 mg/L for ketoconazole. Each *Candida* sp. was tested simultaneously against fluconazole, itraconazole, ketoconazole and voriconazole.

Results and discussion

The fluconazole, itraconazole, ketoconazole and voriconazole MICs for the ATCC isolates were: 0.5, 0.125, 0.06

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and 0.015 mg/L, respectively, for ATCC 90018; 0.5, 0.125, 0.06 and 0.03 mg/L, respectively for ATCC 90028; 0.5, 0.125, 0.03 and 0.03 mg/L, respectively, for ATCC 90029; 16, 0.125, 0.03 and 0.03 mg/L, respectively, for ATCC 90030.

The MIC ranges, MIC_{50}s, MIC_{90}s and geometric mean MICs of ketoconazole, fluconazole, itraconazole and voriconazole for specific *Candida* spp. are presented in Table I. Using the fluconazole breakpoint values proposed by the NCCLS,[8] 69% (46/67) of *Candida* spp. were susceptible, 9% (6/67) dose-dependently susceptible and 22% (15/67) resistant to fluconazole *in vitro*. Using the itraconazole breakpoints,[8] 40% (27/67) were susceptible, 40% (27/67) dose-dependently susceptible and 20% (13/67) resistant to itraconazole *in vitro*.

To our knowledge, this is the first published study to compare the in-vitro efficacy of voriconazole, itraconazole and ketoconazole against a large number of *Candida* spp. with a wide range of fluconazole MICs. We included in our study not only fluconazole-resistant *C. albicans* and *C. krusei*, but also *C. tropicalis*, *C. parapsilosis* and *C. lusitaniae*. We demonstrated that voriconazole was the most potent of the azole agents against the *Candida* spp. tested (geometric mean of 0.12 mg/L), followed by itraconazole (geometric mean of 0.30 mg/L) and ketoconazole (geometric mean of 0.75 mg/L).

Voriconazole had in-vitro activity against both fluconazole-susceptible and -resistant *Candida* spp. For fluconazole-susceptible isolates, voriconazole was significantly more potent than itraconazole and ketoconazole: the geometric mean MIC of voriconazole (0.04 mg/L) was significantly lower than that of itraconazole (0.17 mg/L; $P < 0.001$) and that of ketoconazole (0.43 mg/L; $P < 0.001$) (Table II). Moreover, 91% (42/46) of the fluconazole-susceptible *Candida* spp. exhibited voriconazole MICs of ≤0.125 mg/L, whereas only 52% (24/46) exhibited itraconazole MICs ≤0.125 mg/L, and 48% (21/46) exhibited ketoconazole MICs ≤0.125 mg/L.

For fluconazole-resistant or dose-dependently susceptible isolates, voriconazole also demonstrated good in-vitro activity. Sixty-two percent (13/21) of these isolates exhibited voriconazole MICs of ≤0.5 mg/L, whereas only 43% (9/21) exhibited itraconazole MICs of ≤0.5 mg/L, and 19% (4/21) exhibited ketoconazole MICs of ≤0.5 mg/L. As previously noted, *C. krusei* and *C. glabrata*, species often associated with fluconazole resistance, were sus-

Table I. In-vitro activity of ketoconazole, fluconazole, itraconazole and voriconazole against *Candida* spp.

Species	*n*	Antimicrobial agent	48 h MIC (mg/L)			
			range	50%	90%	geometric mean
C. albicans	24	ketoconazole	0.03–>16	8	>16	1.10
		fluconazole	0.125–>64	0.5	>64	1.30
		itraconazole	0.06–>16	0.125	0.5	0.22
		voriconazole	≤0.015–>16	≤0.015	0.25	0.06
C. tropicalis	17	ketoconazole	0.03–>16	4	>16	1.75
		fluconazole	0.5–>64	8	>64	9.02
		itraconazole	0.015–>16	0.25	>16	0.54
		voriconazole	≤0.015–>16	0.125	>16	0.33
C. glabrata	12	ketoconazole	0.03–1	1	1	0.47
		fluconazole	2–32	8	32	8.00
		itraconazole	0.25–1	0.5	1	0.56
		voriconazole	0.06–0.5	0.125	0.25	0.16
C. parapsilosis	8	ketoconazole	0.03–1	0.125	1	0.19
		fluconazole	0.5–>64	2	32	3.35
		itraconazole	0.125–0.5	0.125	0.25	0.19
		voriconazole	0.015–1	0.03	0.25	0.06
C. krusei	3	ketoconazole	0.5–1	0.5	0.5	0.63
		fluconazole	>64	>64	>64	64.07
		itraconazole	0.25–0.5	0.5	0.5	0.40
		voriconazole	0.5	0.5	0.5	0.50
C. lusitaniae	3	ketoconazole	0.03–0.5	0.03	0.5	0.08
		fluconazole	0.125–32	2	32	1.99
		itraconazole	0.125–0.5	0.125	0.5	0.20
		voriconazole	0.015–0.5	0.015	0.5	0.06

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Table II. Geometric means, MIC_{50} and MIC_{90} for fluconazole-susceptible and -resistant *Candida* species against itraconazole, ketoconazole and voriconazole

	Geometric mean MIC (range) for fluconazole:			MIC_{50}/MIC_{90} (mg/L) for fluconazole:	
Azole agent	susceptible isolates	resistant isolates	*P* value	susceptible isolates	resistant isolates
Itraconazole					
all *Candida* spp.	0.17	1.39	<0.001	0.125/0.5	1/16
C. albicans	0.13	3.97	<0.001	0.125/0.25	16/16
C. tropicalis	0.13	2.51	0.001	0.125/0.25	1/16
C. glabrata	0.46	1.00	0.01	0.5/0.5	1/1
C. parapsilosis	0.18	0.25	NS	0.125/0.25	0.125/0.5
C. krusei	–	0.40	–	–	0.5/0.5
Ketoconazole					
all *Candida* spp.	0.43	2.51	0.003	0.25/16	4/16
C. albicans	0.70	11.36	0.06	0.5/16	8/16
C. tropicalis	1.06	3.06	NS	0.5/16	8/16
C. glabrata	0.37	1.00	NS	0.5/1	1/1
C. parapsilosis	0.11	1.00	0.01	0.125/0.25	1/1
C. krusei	–	0.63	–	–	0.5/0.5
Voriconazole					
all *Candida* spp.	0.04	1.14	<0.001	0.03/0.125	0.5/16
C. albicans	0.02	5.64	<0.001	0.015/0.06	16/16
C. tropicalis	0.08	1.68	0.003	0.06/0.125	0.25/16
C. glabrata	0.13	0.25	NS	0.125/0.25	0.25/0.5
C. parapsilosis	0.03	0.50	0.002	0.03/0.06	0.25/1
C. krusei	–	0.50	–	–	0.5/0.5

NS, not significant.

ceptible *in vitro* to itraconazole,[9–10] to voriconazole[4] with MICs of 0.25–0.5 mg/L, and to ketoconazole with MICs of 0.5–1 mg/L (Table II).

Despite these promising results, there was cross-resistance between fluconazole and voriconazole for some *Candida* spp. For example, isolates with higher fluconazole MICs were associated with higher voriconazole MICs ($P < 0.001$, linear regression). There was also cross-resistance between fluconazole, itraconazole and ketoconazole: the higher fluconazole MICs were associated with higher itraconazole and ketoconazole MICs ($P < 0.001$, and 0.003, respectively). This pattern of cross-resistance has been previously described, and may result from the similar mechanisms of actions of these agents.[9,10]

Six (38%) of the 16 *Candida* spp. with fluconazole MICs of ⩾64 mg/L displayed itraconazole MICs of ⩾16 mg/L and ketoconazole MICs of ⩾8 mg/L. All of these isolates had voriconazole MICs of ⩾16 mg/L. In our study, these high levels of resistance to multiple azole agents (MICs ⩾ 8 mg/L) were seen only for *C. albicans* and *C. tropicalis* isolates. Fluconazole-resistant *C. krusei*, *C. glabrata*, *C. parapsilosis* and *C. lusitaniae* isolates, on the other hand, did not display high-level resistance to itraconazole, ketoconazole and voriconazole.

In conclusion, voriconazole has potent in-vitro activity against *Candida* spp., including those that were dose-dependently fluconazole-susceptible or fluconazole-resistant. This finding suggests that voriconazole might be effective in the treatment of refractory candidosis caused by fluconazole-resistant strains. However, cross-resistance with fluconazole exists in a small subset of *Candida* spp. Given the high oral bioavailability and the well tolerated nature of voriconazole, this drug may become an important addition to the armamentarium of systemic antifungal agents. This promise, however, requires to be confirmed in the clinical setting.

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