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In vitro activity of antifungals against Zygomycetes

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Abstract

To date, no reference standard for therapy for zygomycosis has been established because there are insufficient clinical data with which to make such a judgement. Knowledge of the species responsible for the infection and its antifungal susceptibility profile has become increasingly important in the management of patients. Amphotericin B is the most active drug against all the species involved, followed by posaconazole, whereas voriconazole has no activity. Echinocandins are completely inactive *in vitro*, but may be an interesting option when used in combination with other drugs.

Keywords: Antifungal, in vitro activity, mucormycosis, Zygomycetes

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Introduction

In the last few years, the number of cases of zygomycosis has increased, especially among immunocompromised patients, although several authors have also reported infections in patients with unknown underlying conditions [1–3]. The course of the infection is rapidly progressive and potentially fatal, with high rates of mortality and morbidity. No reference standard for therapy has yet been established. Therapy usually requires a combination of measures, including antifungal treatment, surgical intervention and control of the underlying risk factors [4]. The agent of choice for treating this infection is amphotericin B (AmB) [5]. However, therapy with this drug has produced variable results; toxicity often occurs and the immune status of the patient plays an important role in the outcome, both of which highlight the importance of developing new strategies for treatment. Posaconazole has been used as salvage therapy for zygomycosis and has improved outcome. [6,7]. In addition, echinocandins have been used in combination therapies, underlining the potential utility of other antifungals in the treatment of zygomycosis. The low rates of response to these various therapies can be attributed to a range of factors, but knowledge of the species responsible for the infection and its antifungal susceptibility profile is of increasing value in the management of patients.

Unfortunately, identification by morphology examination of macroscopic and microscopic characteristics requires a high level of expertise. Kontoyiannis *et al.* [8] reported a 20% discrepancy between identification by means of morphology and that achieved by sequencing internal transcribed spacers. In addition, antifungal susceptibility testing data are limited and are based on isolates identified by their morphological characteristics [5,9,10].

The aim of this article is to review the antifungal susceptibility profile of the Zygomycetes in order to provide information for the better management and treatment of the life-threatening infections they cause.

Available Methodologies for Antifungal Susceptibility Testing in Zygomycetes

Two standardized methods are available for determining the susceptibility of moulds to antifungal agents. One method is the CLSI standard 'Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi' (Approved Standard M38-A) [11]. This document recommends the use of: (i) standard RPMI-1640 broth; (ii) non-germinated conidial inoculum suspensions of 10^4 CFU/mL, and (iii) for *Rhizopus* spp., incubation at 35 °C for 24 h. The subcommittee on Antifungal Susceptibility Testing (AFST) of the European Committee for Antimicrobial Susceptibility Testing (EUCAST) has developed an alternative standard for

conidia-forming moulds [12]. The differences with the CLSI methods are: (i) RPMI-1640 is supplemented with glucose to reach a 2% concentration, and (ii) inoculum size is between 1×10^5 and 5×10^5 CFU/mL. Inoculum preparations are performed by means of counting spores in a haematocytometer [13–15]. Concordance between these two methods was studied by Chryssanthou and Cuenca-Estrella [16], who found a level of agreement of 92.5%.

Antifungal Susceptibility Profile of Zygomycetes

Table 1 shows a literature review of the antifungal susceptibility profile of Zygomycetes.

Amphotericin B

Of the antifungal treatments available, AmB shows the best *in vitro* activity against most of the species responsible for zygomycosis (Table 1) [9,17,18]. Unfortunately, these species have a broad range of susceptibilities to this drug [19,20]. *Cunninghamella* spp. and *Rhizopus* spp. have higher minimum inhibitory concentrations (MICs) to AmB, whereas *Mucor* spp. and *Absidia* spp. are subject to greater activity on the part of the drug (Table 1). The highest number of clinical failures relate to infections caused by *Cunninghamella bertholletiae*, which supports the high AmB MICs reported for this Zygomycete [2,21–25]. However, several series and case reports describing successful treatment with this drug have been published [26–37]. A number of retrospective studies have reported an increase in survival rates when lipid formulations of AmB were used as first-line or salvage therapy, primarily liposomal AmB (L-AmB) [26–33]. As these formulations are more effective and better tolerated, they have replaced conventional AmB in the treatment of these infections.

Azole drugs

Azole drugs have a limited *in vitro* activity against Zygomycetes. However, *in vivo* studies with animal models have shown that they can be active against zygomycosis [38,39]. In addition, posaconazole has been used as salvage therapy with positive results, constituting a promising alternative for the treatment of these infections.

Itraconazole

Although many authors have stated that itraconazole is not a good choice for treatment of zygomycosis, some cases of infection have been successfully treated with this drug [40–42]. *In vitro* results show that itraconazole is more active against Zygomycetes than voriconazole and that some strains are inhibited by low concentrations of itraconazole [18,43–45]. *In vitro* studies with itraconazole have shown a wide range of MICs (Table 1) [5,18,46]. Singh *et al.* [46] determined the itraconazole MICs for 15 strains of Zygomycetes, finding that *Rhizomucor*, *Syncephalastrum* and *Mycocladus* (*Absidia*) showed lower MICs of itraconazole (ranges 0.03–2 mg/L), whereas *Cunninghamella* and *Mucor* were more resistant (ranges 0.5 mg/L to >8 mg/L). These data are in accordance with findings reported by Dannaoui *et al.* [5], where *Mycocladus* and *Rhizomucor* were the two genera that showed lower itraconazole MICs. In addition, in a murine model of *Mycocladus corymbifer* infection, itraconazole therapy increased the survival rate of infected animals [47,48]. Therefore, itraconazole may be useful in some cases of zygomycosis in which susceptible strains are involved.

Voriconazole

Voriconazole is not active against Zygomycetes *in vitro*. All studies have shown MICs >2 mg/L. In most studies, MICs >8 mg/L have been reported [5,9,10,18,21,46]. In addition, it has been shown that patients with leukaemia or bone marrow transplantation recipients undergoing voriconazole prophylaxis can develop breakthrough infections caused by Zygomycetes [8, 49, 50].

Posaconazole

Posaconazole is the first drug in the azole drug family to show a broad spectrum of activity against Zygomycetes. *In vitro* studies have shown good activity against these fungi ($MIC_{50} \leq 1$ mg/L) [5,9,10,18]. The species which have shown higher MICs for this drug are *Rhizopus* spp. and *Cokeromyces recurvatus*, with a geometric mean of >2 mg/L, whereas *Absidia* spp. and *M. corymbifer* are the most susceptible species; *Saksenaea vasiformis* and *Rhizomucor* spp. also exhibit low MICs for posaconazole, although few strains have been tested (Table 1). In addition, experimental models of infection have proven the *in vivo* activity of this drug. Among mice treated with posaconazole, a survival increase occurred in mice infected with *Mucor* spp. [51], partial efficacy was seen in those infected with *M. corymbifer*, and a dose-dependent response was found in those infected with *Rhizopus microsporus* [52]. In addition, similar results have been obtained with posaconazole and AmB used as prophylaxis in neutropenic mice [53].

Two clinical studies have evaluated the efficacy of posaconazole as salvage therapy for zygomycosis. Van Burik *et al.* [7] reported a 60% response in 91 patients and Sun *et al.* [51] found a 79% response in 24 patients.

TABLE 1. ***In vitro*** **data of antifungal susceptibility of Zygomycetes to amphotericin B, itraconazole and posaconazole**

			Amphotericin B mg/L				Itraconazole mg/L				Posaconazole mg/L			
Species	***n***	**References**	**Range**	**MIC_{50}**	**MIC_{90}**	**GM**	**Range**	**MIC_{50}**	**MIC_{90}**	**GM**	**Range**	**MIC_{50}**	**MIC_{90}**	**GM**
Rhizopus spp.	15	[5]	0.06–1	0.5	1	0.42	0.25–32	0.5	4	0.87	0.125–1	0.25	0.50	0.27
	101	[9]	0.03–0.5	0.25	0.5		0.03 to >8	0.5	4		0.06–4	0.25	1	
	6	[57]	1–2	1			0.5–2	1						
	10	[18]	0.06–2	0.125	0.5	0.33	0.25–8	1	8	3.93	0.25–8	1	8	2.73
R. microsporus	12	[9]	0.03–0.5	0.25	0.25		0.25–1	0.5			0.25–2	0.25		
	1	[9]	0.25				1							
	1	[61]	0.25				>16							
	5	[21]	1–2	1	2		8 to >8	>8	>8					
R. oryzae	14	[21]	0.25–8	1	4		0.5 to >8	>128	>128					
	2	[46]	0.03–0.06			0.04	0.25–4			1.41				
	20	[9]	0.06–0.5	0.25	0.25		0.25–2	0.5			0.03–1	0.25	1	
		[43]	0.12–0.25	0.25	0.25		0.5–4	1	2					
	15	[10]		0.5	2			>8	>8			0.5	8	
Mucor spp.	6	[5]	0.03–0.25	0.125		0.09	1–32	8		6.96	0.5–2	1		1.15
	41	[9]	0.125–4	0.25	0.5		0.25 to >8	0.5	>8		0.06–2	0.5	2	
	6	[18]	0.06–0.5	0.25	0.25	0.24	0.25–8	1	2	2.18	0.125–8	0.5	1	1.54
M. circinelloides	6	[9]	0.06–0.5	0.25			2 to >8				1–2			
	1	[21]	0.25				8							
	3	[46]	0.03			0.03	0.5 to >8			2.82				
M. ramosissimus	3	[21]	0.12–0.5	0.25	0.5		1–8	2	8					
Absidia spp.	3	[9]	0.25–0.5				0.5–1				0.125			
	10	[5]	0.06–0.125	0.125	0.125	0.09	0.03–0.125	0.06	0.25	0.08	0.06–0.25	0.06	0.125	0.09
Mycocladus corymbifer	9	[9]	0.25–0.5	0.25			0.125–0.5				0.06–0.25			
	5	[18]	0.25–0.5	0.25	0.25	0.30	0.03–0.25	0.06	0.25	0.14	0.03–0.25	0.03	0.25	0.13
	4	[21]	0.25 to >16	0.50	>16		1 to >8	2	>8					
	3	[46]	0.03–0.25			0.05	0.125–2			0.62				
	1	[61]	0.06				0.03							
		[43]	0.06–0.25	0.25	0.25		0.25–0.5	0.25	0.5					
Rhizomucor spp.	3	[5]				0.06				0.09				0.09
	5	[9]	0.125–0.25	0.125			0.125–1				0.06–1			
R. pusillus	3	[46]	0.125–0.25			0.16	0.03–0.125			0.07				
Cunninghamella spp.	13	[9]	0.25–2	1			0.125–4	1			0.06–1	0.5		
	5	[18]	0.125–2	0.25	0.25	0.55	0.125–2	0.25	0.5	0.60	0.03–1	0.25	1	0.36
C. bertholletiae	1	[5]				2				1				0.5
	1	[21]	4				2							
	2	[46]	0.25–0.5			0.35	1–4			2				
Aphophysomyces elegans	6	[9]	0.03–1	0.125			0.03–4	0.125			<0.016–1	0.03		
	1	[5]				2				0.5				0.5
	4	[18]	0.03–1	0.03	0.25	0.33	0.03–8	0.5	2	2.63	0.03–4	0.25	2	1.57
	1	[61]	2				0.5							
Saksenaea vasiformis	4	[18]	0.125–2	0.125	0.25	0.23	0.015–0.03	0.015	0.03	0.05	0.015–0.25	0.06	0.125	0.11
	1	[21]					0.01							
Cokeromyces recurvatus	2	[18]	0.125–2	0.125	2	0.31	0.25–8	0.25	8	4.13	0.25–4	0.25	4	2.13
Syncephalastrum racemosum	2	[46]	0.03			0.03	0.03–0.25			0.1				

n, number of isolates per species; MIC_{50}, MIC causing inhibition of 50% of isolates; MIC_{90}, MIC causing inhibition of 90% of isolates; GM, geometric mean.
A blank space means no data.

Finally, some case reports of successful treatment of patients with zygomycosis have also been published [54–56], highlighting posaconazole as a promising drug for treatment of these infections.

Echinocandins

Echinocandins have been reported as inactive *in vitro* against Zygomycetes [44,46,57]. Caspofungin has been tested against 217 strains [9], all of which were resistant *in vitro* (MICs >16 mg/L). Singh *et al.* [46] found caspofungin to have no activity in a collection of 15 Zygomycetes (MICs >16 mg/L). Kontoyiannis *et al.* [8] also studied the *in vitro* activity of caspofungin against 20 Zygomycetes with similar results (MICs >32 mg/L). However, murine models of zygomycosis [9,58–60] have shown that echinocandins may enhance the activity of AmB in the treatment of these infections. Therefore, echinocandins have potential use when combined with other antifungal drugs.

Terbinafine

Few studies have analysed the activity of terbinafine against Zygomycetes. Dannaoui *et al.* [5] tested terbinafine against 36 Zygomycetes isolates, obtaining a wide range of MICs (Table 1). Terbinafine was active against all isolates of *M. corymbifer* and some *Rhizopus* and *Mucor* isolates. Interestingly, *R. microsporus* was susceptible to the drug, whereas *Rhizopus oryzae* was not.

Combination Therapy

The management of these infections is difficult because of the limited number of drugs active against the causative agents of zygomycosis. Several studies have analyzed the *in vitro* activity of antifungals in combination against Zygomycetes. Dannaoui *et al.* [61] tested 35 isolates of Zygomycetes and found

synergistic effects between terbinafine + AmB and terbinafine + voriconazole (in 20% and 44% of isolates, respectively). Gomez-Lopez *et al.* [62] evaluated the *in vitro* combinations of terbinafine with itraconazole or AmB against 17 clinical isolates of Zygomycetes and found that terbinafine + itraconazole exhibited a synergistic effect in 82% of isolates, especially for *R. microsporus*, *M. corymbifer* and *C. bertholletiae*, as did terbinafine + AmB in 53% of isolates.

Animal models have shown that the interaction between AmB and caspofungin or posaconazole improves survival in mice, indicating a synergistic effect between these drugs [59,63]. Sugar and Liu [39] reported a synergistic effect for the combination of azole drugs and quinolones in mice with pulmonary mucormycosis.

In addition, caspofungin combined with AmB was more successful than AmB alone in treating patients with rhino-orbital-cerebral mucormycosis [60] and this combination was also used successfully to treat a case of rhinocerebral zygomycosis in a haematological cancer patient [64].

Conclusions

Zygomycetes are a heterogeneous group of fungi with a wide antifungal susceptibility profile. Amphotericin B is the agent of choice to treat zygomycosis. However, its toxicity remains a problem and therefore alternative therapies are needed, including, for example, lipid formulations of AmB. Posaconazole is the second most active agent against these fungi and has shown good results *in vitro*, in animal models and also in patients. Combination therapies with azoles or echinocandins may also represent alternatives to improve the survival of patients infected with Zygomycetes.

Transparency Declaration

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