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Posaconazole Enhances the Activity of Amphotericin B against *Aspergillus* Hyphae In Vitro▽

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The MICs and fractional inhibitory concentrations of posaconazole (POS) and voriconazole (VRZ), alone and in combination with amphotericin B (AMB), for the conidia and hyphae of 100 *Aspergillus* isolates were evaluated. POS-AMB had more synergistic activity against hyphae (75% of isolates) than VRZ-AMB (37%) and significantly more synergistic activity against hyphae than against conidia (12%).

Filamentous fungal pathogens are recognized as major and increasing sources of infection in immunocompromised hosts (6, 18). The most common species causing disease in patients is *Aspergillus fumigatus* (90%), followed by *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus terreus* (6, 8). In the meantime, besides amphotericin B (AMB), several other drugs are available as treatments for invasive aspergillosis, such as voriconazole (VRZ), posaconazole (POS), and caspofungin (1, 9, 23, 24). POS was recently approved for use for the treatment of patients with invasive infections that are refractory to other antifungal agents (17). The high rate of mortality from mold infections and the relatively limited efficacies of the current agents have produced a significant interest in the use of polyene- and azole-based combinations for these difficult-to-treat infections (3, 5, 11, 19). The present study evaluated the antifungal activity of either POS or VRZ, alone and in combination with AMB, against the conidia and hyphae of *Aspergillus* spp. in vitro.

We tested 25 clinical isolates each of *A. fumigatus*, *A. terreus*, *A. flavus*, and *A. niger* from patients with invasive aspergillosis. The MICs of AMB (Sigma Aldrich, Vienna, Austria), VRZ (kindly provided by Pfizer, Vienna, Austria), and POS (kindly provided by Schering-Plough Research Institute, Kenilworth, NJ) for *Aspergillus* spp. were tested according to the guidelines described in the CLSI (formerly the NCCLS) M38-A document (15). The MICs for hyphae were tested by the method of Lass-Flörl et al. (12). For all drugs the endpoints were read at 100% inhibition of conidia germination and hyphal growth after 48 h of incubation at 35°C. The endpoints read at 80% resulted in similar MIC data (not shown). Drug combinations were assessed by a checkerboard method. The synergy tests were evaluated by using the MIC endpoints of each drug. The fractional inhibitory concentration (FIC) of each drug for an individual isolate was calculated as the ratio of the concentration of the drug in combination that achieves the MIC endpoint to the MIC of the drug alone obtained by use of that endpoint. FIC index values were interpreted as follows: FIC ≤ 0.5, synergistic; FIC ≥ 1 to ≤ 4, indifferent; and FIC >4, antagonistic (3). Also, the metabolic activity of drug-treated hyphae was determined by their ability to reduce the tetrazolium compound 3-(4,5-dimethl-2-thiazol)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), as described elsewhere (13). Duplicate testing was performed on separate days. The results of the in vitro tests were compared by the log-rank test. Significance was defined as a P value of <0.05.

The MIC ranges for conidia and hyphae of *Aspergillus* spp. are given in Table 1, and the interaction results are given in Table 2. The POS-AMB and VRZ-AMB interactions were synergistic to indifferent. The activity of the combination of POS and AMB was significantly more synergistic against hyphae than against conidia. Visual readings of growth inhibition were correlated with the colorimetric assessments of the metabolic activities of fungi. For hyphae, comparison of the visually determined endpoints with the results of the MTT method revealed that 84.7% of the visually determined MICs corresponded to a 95% or greater reduction in metabolic activity, as measured by determination of the optical density.

Our MIC data for POS and VRZ for conidial suspensions of *Aspergillus* spp. were comparable to those presented in previously published reports (3, 7, 9, 14, 22). The in vitro activities of POS in combination with AMB against conidia of *Aspergillus* ranged from indifferent (88% of isolates) to

TABLE 1. MIC results for the various *Aspergillus* spp. tested

Drug	*Aspergillus* sp.	MIC_{90} (μg/ml)	
		Conidia	Hyphae
POS	*A. fumigatus*	0.125	0.5
	A. terreus	0.25	1
	A. flavus	0.125	0.5
	A. niger	0.25	1
VRZ	*A. fumigatus*	0.125	1
	A. terreus	0.5	1
	A. flavus	0.5	2
	A. niger	0.5	2
AMB	*A. fumigatus*	0.625	2.5
	A. terreus	2.5	5
	A. flavus	1.25	2.5
	A. niger	1.25	5

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TABLE 2. FIC results for the various *Aspergillus* spp. tested

Effect	Drug combination	FIC (% of isolates)	
		Conidia	Hyphae
Synergistic	POS-AMB	12	75[a]
	VRZ-AMB	43	37
Indifferent	POS-AMB	88	25
	VRZ-AMB	57	63

[a] $P < 0.05$.

synergistic (12% of isolates). The activity of the VRZ-AMB combination was more equally distributed between indifferent and synergistic (57% and 43%, respectively). The results of a number of in vivo and in vitro studies with the POS-AMB and VRZ-AMB combinations have been published, and all studies showed a lack of antagonism (5, 14, 19, 21). These data and those from our study suggest evidence of a possible additive and/or synergistic effect. However, antifungal combination therapies are still controversial (16, 19). Some studies have suggested that azole antifungal agents would antagonize the effects of AMB (16, 20). Also, in vitro combination tests must evaluate complex events that are difficult to assess (19). Using the same combinations, different authors have observed a spectrum from antagonism to synergism, depending on the methodology and analysis used (16, 19). However, the antagonism of the POS-AMB and VRZ-AMB combinations has not been reported so far.

The onset of invasive *Aspergillus* infection is associated with the appearance of hyphae (10). Consequently, an agent must be active against the hyphal form in order to be clinically effective. POS and VRZ exerted strong activities against the hyphae of *Aspergillus* spp.; and the POS-AMB combination yielded excellent results, as the FIC indices were synergistic for 75% of isolates. These FIC indices were significantly ($P < 0.05$) higher than those for synergistic activity against the conidia (12%). The underlying mechanism for this effect is unknown. Differences in the sterol compositions, the fungal cell membrane transporters, and the cell wall compositions of hyphae and conidia could account for this finding (2). One possible explanation for the synergy could be that polyene (AMB) binding to the fungus destabilizes the membrane and facilitates the entry of the azole (POS or VRZ). Our results suggest that a combination of AMB with POS might be effective against infections due to *Aspergillus* spp., as shown by Najvar et al. (14).

Clinical studies confirm that POS has a favorable safety profile during treatment of seriously ill patients with invasive fungal infections (3, 9, 17). POS appears to be well tolerated (9, 17), making this substance a promising candidate for use for the prevention and treatment of fungal infections in immunocompromised patients. The lack of antagonism in vitro and in vivo (3, 4, 14, 21) suggests that POS-AMB and VRZ-AMB may be used as combination therapies for the treatment of fungal infections.

In conclusion, POS and VRZ exhibited excellent in vitro activities against the hyphae of *Aspergillus* spp., and the combination of POS and AMB was significantly more active against hyphae than against conidia ($P < 0.05$). Further synergy tests with this drug combination are warranted, and the impact of the combination on patient outcomes needs to be further investigated.

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