

## Synergistic Effect of Posaconazole and Caspofungin against Clinical Zygomycetes<sup>▽</sup>

The use of echinocandins for the treatment of invasive zygomycosis (IZ) is controversial since they show no activity in vitro. Although posaconazole shows promising results, the mortality rates of patients with IZ treated with posaconazole, at 30%, remain unacceptably high. The use of new therapies with alternative agents alone or combined is mandatory.

This is the first study to evaluate the in vitro effect of combining posaconazole (Schering-Plough, Kenilworth, NJ) and caspofungin (MSD, Merck Research Laboratories, Rahway, NJ) against 12 clinical zygomycetes. Zygomycetes were identified by conventional morphological methods (8).

Drug combinations were tested by using a checkerboard modification of the guidelines presented in CLSI document M38-A. The final concentrations of posaconazole and caspofungin were 0.06 to 4 µg/ml and 0.25 to 256 µg/ml, respectively. The adjusted suspensions of sporangiospores had an optical density of 0.15 to 0.17 (68 to 70% transmittance). The MIC for each drug alone and its combinations was defined as the lowest concentration that produced complete visual inhibition of fungal growth (6). Drug interactions were defined as synergistic if the lowest fractional inhibitory concentration index was ≤0.5 (6). Although the caspofungin MICs were all above serum drug levels, the combination proved to be synergistic in all strains (Table 1).

Caspofungin exhibits minimal activity against zygomycetes when tested in vitro, although this information should be interpreted with caution (10). The mechanisms underlying the lack of activity of echinocandins against zygomycetes are believed to be attributable to differences in their cell wall compositions, as these organisms largely contain 1,3-α-glucan and glycuronomannoproteins instead of 1,3-β-D-glucan. However,

*Rhizopus oryzae* expressed the target enzyme for caspofungin (FKS1), and this drug presented limited activity in a murine model of disseminated IZ (3).

Combinations of two drugs have been studied mainly for the treatment of cryptococcal meningitis (5). The combination of caspofungin and posaconazole has been evaluated in other fungi and shows synergy or indifference but never antagonism (1, 7). The combination of two antifungal agents has been shown to be effective against zygomycetes in vitro and in vivo (2). In animal models, the combination of caspofungin and amphotericin B lipid complex was synergistic, improving the survival of mice (9). Few cases of IZ responded partially to the echinocandins, although we have so little clinical experience with caspofungin for the treatment of IZ (4, 11). These data suggest that echinocandins may have a role as a second agent in combinations.

The precise mechanism of synergy found between posaconazole and caspofungin must be shown. A possible explanation may be that the inhibition of the synthesis of ergosterol by posaconazole may be changing the membrane environment and making the enzyme FKS1 more accessible or sensitive to inhibition by caspofungin. The result is that the active agent posaconazole is potentiating the activity of caspofungin. Another possibility is that the effect of caspofungin interfering with the structure of the cell wall may facilitate the penetration of posaconazole into the cell and make this agent more active.

However, the clinical value of these in vitro data is unknown. In vitro-in vivo correlation is needed for this combination.

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TABLE 1. In vitro activities of posaconazole and caspofungin alone and in combination against 12 zygomycete isolates obtained by a checkerboard modification of the CLSI M38-A procedure

Zygomycete strain no.	MIC (µg/ml) of drug:			Lowest FICI <sup>a</sup> for combination
	Alone		In combination (caspofungin/posaconazole)	
	Caspofungin	Posaconazole		
<i>Rhizopus</i>				
0	>256	1	32/0.25	0.312
3	>256	1	16/0.25	0.281
8	>256	1	8/0.25	0.265
<i>Absidia</i>				
11	>256	1	4/0.25	0.258
17	256	1	16/0.25	0.281
18	>256	0.5	64/0.125	0.375
<i>Cunninghamella</i> sp. strain 22	>256	1	128/0.25	0.5
<i>Snycephalastrum</i> sp. strain 23	>256	1	8/0.125	0.187
<i>Rhizomucor</i> sp. strain 25	128	2	32/0.5	0.375
<i>Mucor</i>				
28	256	0.5	32/0.125	0.375
39	>256	1	64/0.125	0.25
43	256	0.5	32/0.125	0.375

<sup>a</sup> Fractional inhibitory concentration index.

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