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Caspofungin Resistance in *Candida albicans*: Correlating Clinical Outcome with Laboratory Susceptibility Testing of Three Isogenic Isolates Serially Obtained from a Patient with Progressive Candida Esophagitis

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A patient with azole-refractory thrush-esophagitis responded initially to caspofungin, but the treatment eventually failed. In a murine model, caspofungin was effective against two early isolates for which the MICs of caspofungin were low, but it was less effective against a late isolate for which the MIC of caspofungin was greater. We concluded that there is a correlation between in vivo failure and rising in vitro caspofungin MICs.

The National Committee for Clinical Laboratory Standards (NCCLS) has generated standardized in vitro testing methods for triazoles (9). Fluconazole is currently the drug of choice for treatment of mucosal and systemic *Candida* infection (13). However, isolates of *Candida albicans* and *C. glabrata* for which the fluconazole MICs are ≥64 μg/ml have been associated with clinical failure of fluconazole therapy (14, 15). However, it is unclear whether results of in vitro testing can be generalized from triazoles to other antifungals such as the echinocandins. These compounds inhibit fungal cell wall synthesis by blocking formation of 1,3-β-D-glucans (2, 5; K. Bartizal, M. Motyl, P. Hicks, C. Sable, M. DiNubile, and N. Kartsonis, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-1240, 2002). Caspofungin, a licensed echinocandin, acts rapidly against *Candida* spp. and is considered by some to be fungicidal in vitro (3). The MIC of caspofungin is very low (≤1 μg/ml) for virtually all *C. albicans* isolates. Caspofungin is as effective as fluconazole in the treatment of thrush and esophagitis (1). Patients with esophagitis caused by fluconazole-resistant *C. albicans* isolates have also responded to caspofungin (C. Kutler, B. Koll, B. Raucher, and B. Saltznab, 40th Annu. Meet. IDSA, abstr. 350, 2002).

In contrast to *C. albicans*, caspofungin MICs of ≥4 μg/ml have been reported for *C. parapsilosis* (8). In a large in vitro survey, Pfaller et al. found that only 60% of 420 *C. parapsilosis* isolates showed susceptibility to caspofungin at 1 μg/ml versus >96% of more than 3,000 isolates of the other species tested, including 99% of 2,453 *C. albicans* isolates (11). However, when caspofungin was used for the treatment of patients with candidemia, those with *C. parapsilosis* fungemia (14 of 20) responded as well as those with *C. albicans* and other species (13 of 20) (8). Therefore, the correlation of in vitro susceptibility and clinical response, at least for *C. parapsilosis*, does not seem as close for caspofungin as for fluconazole (4, 6, 12). Additionally, pharmacokinetic considerations such as drug access to sites of infection, distribution in tissue, or clearance may contribute to clinical failure. Thus, it is not clear what role MIC testing has in the prediction of the success or failure of caspofungin therapy. The patient reported in this study allowed us to examine the relationship of a rising in vitro caspofungin MIC and clinical outcome of *C. albicans* esophagitis.

A patient with AIDS (CD4 count of 32 cells/ml of blood) presented with thrush and esophagitis on day 1. Previous therapy with fluconazole at 200 mg twice per day (BID) for 24 weeks and amphotericin B lipid complex at 200 mg BID for 1 week had failed. Esophagoscopy confirmed grade 1 *Candida* esophagitis. Following cultures, he was treated with a loading dose of 70 mg of caspofungin, followed by 50 mg/day (intravenously) for 28 days. His thrush improved at day 6 and was cleared at day 13. His esophagitis responded symptomatically, and later esophagoscopy at 25 days of therapy showed grade 0. Therapy was stopped at 28 days, and the thrush and symptoms of esophagitis returned 22 days later. Caspofungin was reinitiated. The patient improved, but his dense confluent plaques did not resolve completely. The therapy eventually failed, at which time caspofungin was combined with amphotericin B lipid complex at 2 mg/kg. There was no significant improvement on this regimen, and the therapy was declared a failure after 2 months.

During this period, *C. albicans* isolates were recovered from the oral cavity of the patient. Isolate 1 was obtained on day 1, which was prior to caspofungin administration. Isolate 2 was obtained on day 511 after isolate 1. This is 50 days after the first course of successful treatment began. Isolate 3 was obtained on day 553 after isolate 1. This is after clinical failure. Strain identity was investigated by karyotyping, restriction fragment length polymorphism, fingerprinting analysis with moderately repetitive probe Ca3, and sequencing of ERG11 genes as described previously by our group (7, 10). DNA typing and microsatellite locus sequencing methods cannot definitively guarantee that strain 3 arose from strain 2. However, all of

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TABLE 1. Fungal burdens in kidneys of mice infected with *C. albicans*

Isolate	Caspofungin MIC (μg/ml)		Inoculum size (CFU/mouse [10^5])	Median (range) $\log_{10}$ no. of CFU/pair of kidneys						
	RPMI medium	AM3		Control	Caspofungin at:					Fluconazole at 5 mg/kg BID
					0.0625 mg/kg	0.125 mg/kg	0.25 mg/kg	0.5 mg/kg	1 mg/kg	
1	0.25	<0.125	2.9	5.8 (5.5–7.4)	5.1 (2.6–5.8)[a]	3.9 (0–5.1)[a]	3.8 (2.4–6.2)[a]	2.6 (1.6–2.9)[a]	2.9 (2.4–3.6)[a]	5.9 (5.5–6.5)
2	0.25	<0.125	6	5.6 (2.4–6.4)	5.6 (2.4–6.4)	2.4 (1.9–3.6)[a]	2.1 (2.0–2.9)[a]	1.9 (1.4–3.4)[a]	1.7 (1.7–5.2)[a]	6.0 (5.7–7.3)
3	>64	0.5	3–5.5[b]	5.4 (3.6–6.3)	5.4 (3.6–6.3)	5.3 (3.0–6.3)	5.2 (4.3–6.2)	4.9 (3.8–5.6)	4.4 (3.6–5.5)[a]	5.5 (2.6–6.3)

[a] $P \leq 0.05$ (significant reduction) compared with controls.
[b] Pooled studies.

these methods strongly suggested that these three isolates were isogenic, and thus the results strongly support the development of resistance in the same strain.

By the NCCLS method with RPMI 1640 medium, the fluconazole MIC for all three isolates at 48 h of incubation was >64 μg/ml. The caspofungin MICs for isolates 1 and 2 at 48 h were 0.25 μg/ml in RPMI 1640 medium and 0.125 μg/ml in antibiotic medium 3 (AM3). The caspofungin MIC of isolate 3 was >64 μg/ml in RPMI 1640 medium and 0.5 μg/ml in AM3 at 48 h. Thus, the results of susceptibility testing indicated rising MICs due to caspofungin treatment in the same infecting *C. albicans* strain. The rise in the MIC was much greater in RPMI medium than in AM3. The NCCLS has, in general, used RPMI 1640 medium as the test medium, although AM3 is preferred for amphotericin B MIC testing. RPMI medium shows trailing with some *Candida* strains, although this was not a problem with our isolates. No method has been adopted as a standard for caspofungin MIC testing.

For in vivo studies, the *C. albicans* isolates were cultured in brain heart infusion broth at 37°C for 24 h before infection. ICR mice were inoculated intravenously with a 0.2-ml volume. The inoculum of viable organisms was determined by serial dilution of quantitative cultures. Therapy began 1 day after infection and continued through day 7. Caspofungin was given intraperitoneally at 0.0625, 0.125, 0.25, 0.5, and 1 mg/kg/day. Fluconazole was given orally by gavage at 5 mg/kg BID. Controls received sterile water orally by gavage. For any mice succumbing before day 8, the kidneys were removed, weighed, and homogenized for quantitative determination of fungal counts in the tissue. All surviving mice were terminated on day 8, and organs were removed for determination of fungal counts in tissues. The Mann-Whitney test, (with $P \leq 0.05$ necessary for significance) was used to compare differences in tissue burdens between the treatment groups and controls. The results, shown in Table 1, indicate that treatment with fluconazole at 5 mg/kg BID did not reduce the counts of any isolate in kidney tissue. Treatment with caspofungin at 0.0625 to 1 mg/kg significantly reduced the counts of isolate 1 in kidney tissue. Treatment with doses of 0.125 to 1 mg/kg reduced the counts of isolate 2 in kidney tissue. However, caspofungin at 0.0625 mg/kg did not reduce counts ($P \geq 0.05$). Caspofungin reduced the counts of isolate 3 in kidney tissue only at 1 mg/kg, while doses ranging from 0.5 to 0.625 mg/kg failed to do so.

In summary, an elevated in vitro MIC in both RPMI medium and AM3 correlated with a clinical outcome of reduced susceptibility to caspofungin in this study of one patient, as well as in a mouse model of disseminated candidiasis.

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