Serum Differentially Alters the Antifungal Properties of Echinocandin Drugs[▽]

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Antifungal efficacies of the echinocandin drugs caspofungin, micafungin, and anidulafungin were reduced significantly in the presence of 50% human serum, which yielded nearly equivalent MICs or minimum effective concentrations against diverse *Candida* spp. and *Aspergillus* spp. Consistent with a direct drug interaction, serum decreased the sensitivity of glucan synthase to echinocandin drugs.

The echinocandin drugs caspofungin, micafungin, and anidulafungin inhibit the fungal β-1,3-glucan synthase enzyme, which blocks the formation of glucan polymers, thereby disrupting fungal cell wall integrity (2). Animal and human studies indicate that echinocandin drugs are extensively bound to serum proteins (1, 4, 5, 15), and serum was shown to reduce the antifungal properties of micafungin with some *Candida* spp. (3), yet little is known about the influence of serum on antifungal efficacy with the different echinocandin drugs.

Echinocandin susceptibility in the presence or absence of 50% human serum (Sigma) was evaluated with a diverse collection of clinical isolates, laboratory strains, and reference strains of *Candida* spp. and *Aspergillus* spp., according to the guidelines in CLSI documents M27-A2 (9) and M38-A (8), respectively. Abnormal colony morphology was used to establish a minimum effective concentration (MEC) for *Aspergillus* spp. after 48 h of incubation at 35°C (6). Glucan synthase (GS) isolation and 50% inhibitory concentration (IC_{50}) inhibition kinetics were performed as described previously (11). A murine candidiasis model utilizing female BALB/c mice (age, 10 to 12 weeks; weight, 20 to 25 g) was used to assess the relative in vivo efficacies of echinocandin drugs (13).

Serum increased caspofungin MICs an average of 2-fold, with a range of 1- to 16-fold, while it had a more pronounced effect on the other drugs, increasing the MIC an average of 16-fold with a range of 8- to 256-fold for anidulafungin and an average of 64-fold with a range of 32- to 128-fold for micafungin (Table 1). The effects of serum on MICs were assessed for other non-*Candida albicans* spp. The largest MIC shift for caspofungin (eightfold) was with *Candida krusei*, while *Candida tropicalis* strains showed the most significant shifts (128-fold) for both micafungin and anidulafungin. These drugs consistently showed pronounced shifts, which reflected their greater antifungal potencies in the absence of serum. These differences disappeared in the presence of 50% serum, where all three drugs showed comparable MICs.

Similar serum-induced effects were observed with a collection of *Aspergillus* spp., where microscopically observed MECs for the three drugs shifted higher in the presence of serum. Micafungin and anidulafungin again showed the most pronounced antifungal shifts, 32- to 133-fold and 16- to 32-fold, respectively, reflecting their more active behavior in the absence of serum (Table 2). The three drugs displayed nearly equivalent MECs in the presence of 50% serum.

The reduced antifungal properties of echinocandin drugs in the presence of serum suggested that protein binding was having a direct effect on the drugs, perhaps by altering their ability to inhibit glucan synthase. This possibility was investigated by examining direct effects of serum in inhibition of glucan synthase from *C. albicans* and *Aspergillus fumigatus*. As illustrated for anidulafungin and caspofungin, increasing amounts of serum (0 to 50%) decreased drug effectiveness (higher IC_{50}s) (Fig. 1). Similar behavior was observed with enzymes isolated from three different strains of *A. fumigatus* and *C. albicans* (Table 3). The average IC_{50} n-fold shifts for the enzymes at maximum serum levels for caspofungin, micafungin, and anidulafungin were 12.8 ± 6.3, 4.6 ± 0.7 and 11.3 ± 9.5 ng/ml, respectively, for *A. fumigatus* and 5.2 ± 0.9, 4.9 ± 1.1 and 10.5 ± 4.2 ng/ml, respectively, for *C. albicans*. The data suggest that serum exerts its effect through a direct drug interaction. The GS IC_{50} shift for caspofungin was consistent with serum-induced shifts in the MIC or MEC (Tables 1 and 2). The larger MIC shifts for micafungin and anidulafungin suggested a secondary factor might play a role, such as drug transport into the cell. This suggestion is supported by data showing that all three drugs inhibited GS in the same drug range (IC_{50}) (Table 3). In the absence of serum, the enhanced antifungal potencies of micafungin and anidulafungin would reflect better drug penetration into the cell and access to the target. Efficient uptake for these drugs would be expected given their highly hydrophobic tail structures. Furthermore, the drugs bound to serum proteins may be presented as altered substrates for a high-affinity transporter operating at or near the MIC (10).

The effect of serum in neutralizing MIC differences was also consistent with results of animal model studies in which caspofungin and anidulafungin were comparable in efficacy while micafungin was only somewhat less effective over the dose range (0.05 to 1 mg/kg of body weight/day) in reducing kidney

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TABLE 1. Effect of 50% serum on echinocandin MICs[a] of *Candida* spp.

Candida sp. (n[b])	Caspofungin			Anidulafungin			Micafungin		
	MIC$_{50}$		Fold change	MIC$_{50}$		Fold change	MIC$_{50}$		Fold change
	−Serum	+50% serum		−Serum	+50% serum		−Serum	+50% serum	
C. albicans[c] (47)	≤0.25 (0.0156–0.5)	0.5 (0.03–2.0)	2 (1–16)	0.0156 (0.0156)	0.25 (0.125–4.0)	16 (8–256)	0.0156 (0.0156)	1 (0.5–2.0)	64 (32–128)
C. glabrata[c]	0.5 (0.25–0.5)	1 (1.0–2.0)	4 (2.0–4.0)	0.0156 (0.0156–0.125)	1 (0.5–2)	64 (16–64)	0.0156 (0.015)	1 (1)	64 (64)
C. krusei (10)	≤1 (0.25–2.0)	8 (0.5–16.0)	8 (0.5–16.0)	0.25 (0.0156–0.25)	8 (2.0–16.0)	32 (16–256)	0.06 (0.015–0.06)	8 (2.0–16.0)	133 (8–267)
C. parapsilosis (10)	1 (0.5–2.0)	4 (2–16.0)	4 (2–8)	2 (1–4)	16 (16)	8 (4–16)	1 (0.125–2.0)	16 (16)	16–128 (8–128)
C. tropicalis (10)	0.125 (0.015–0.25)	0.5 (0.125–0.5)	4 (1–33)	0.0156 (0.0156–0.031)	2 (0.125–2.0)	128 (8–128)	0.0156 (0.0156)	2 (0.5–4.0)	128 (8–256)

[a] All values represent averages for triplicate experiments with less than 15% variance. Numbers in parentheses represent n-fold changes relative to values for the zero-serum control.
[b] n, no. of isolates.
[c] *C. glabrata* strains grew poorly in human serum. Therefore, mouse serum (Pel-Freez, Rogers, AR) was used. −Serum, no serum.

TABLE 2. Effect of 50% serum on caspofungin and micafungin MECs[a] of *Aspergillus* spp.

Aspergillus sp. (n[b])	Caspofungin			Anidulafungin			Micafungin		
	MIC$_{50}$		Fold change	MIC$_{50}$		Fold change	MIC$_{50}$		Fold change
	−Serum	+50% serum		−Serum	+50% serum		−Serum	+50% serum	
A. fumigatus (10)	<0.25 (0.016–0.25)	1 (0.5–2.0)	11 (2–67)	0.0156 (0.016)	0.5 (0.25–2.0)	32 (32–128)	0.0156 (0.0156)	1 (0.5–2.0)	64 (33–133)
A. flavus (7)	0.03 (0.016–0.06)	0.5 (0.125–0.5)	8 (4–17)	0.0156 (0.016)	0.5 (0.125–1.0)	32 (16–64)	0.0156 (0.0156)	0.5 (0.5–2.0)	33 (17–33)
A. niger (8)	0.06 (0.06–0.25)	1.0 (0.5–1.0)	8 (4–17)	0.0156 (0.0156)	0.5 (0.5–0.5)	32 (16–32)	0.0156 (0.0156)	2 (0.5–2.0)	133 (33–133)
A. terreus (9)	0.125 (0.03–0.5)	0.5 (0.25–0.5)	4 (1–8)	0.0156 (0.0156)	0.25 (0.125–0.25)	16 (8–16)	0.0156 (0.0156)	0.5 (0.125–0.5)	32 (4–32)

[a] MEC is defined as the lowest concentration of drug producing a macroscopically visible morphological change. For an explanation of values, see footnote a of Table 1.
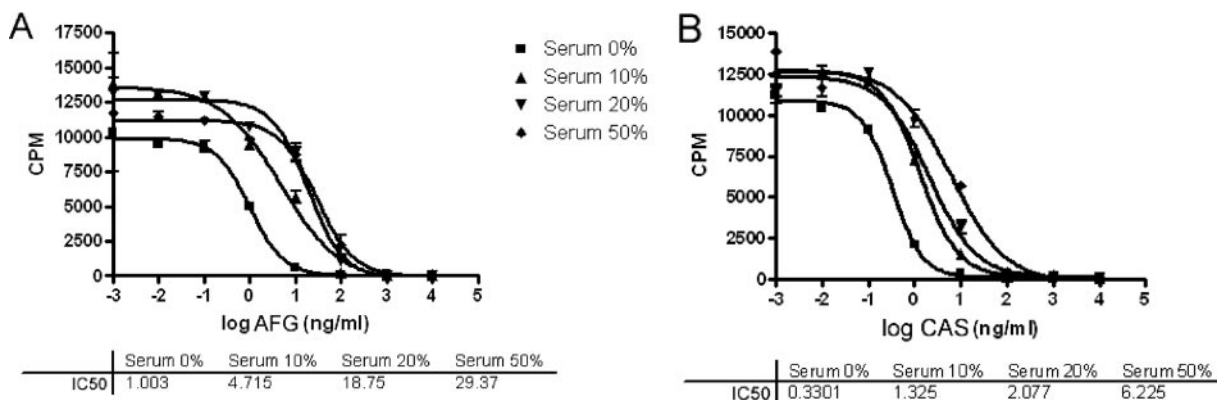[b] n, no. of isolates.

FIG. 1. Serum alters the IC_{50} for glucan synthase from *C. albicans* by anidulafungin and caspofungin. Drug inhibition kinetics for glucan synthase from *C. albicans* strain 36082 were determined by monitoring the incorporation of [³H]uridine diphosphoglucose (CPM) as a function of anidulafungin (AFG) (A) or caspofungin (CAS) (B) concentration in the presence of increasing amounts of serum, as indicated. The calculated IC_{50}s are shown for each drug at a given serum level. All measurements were performed in triplicate.
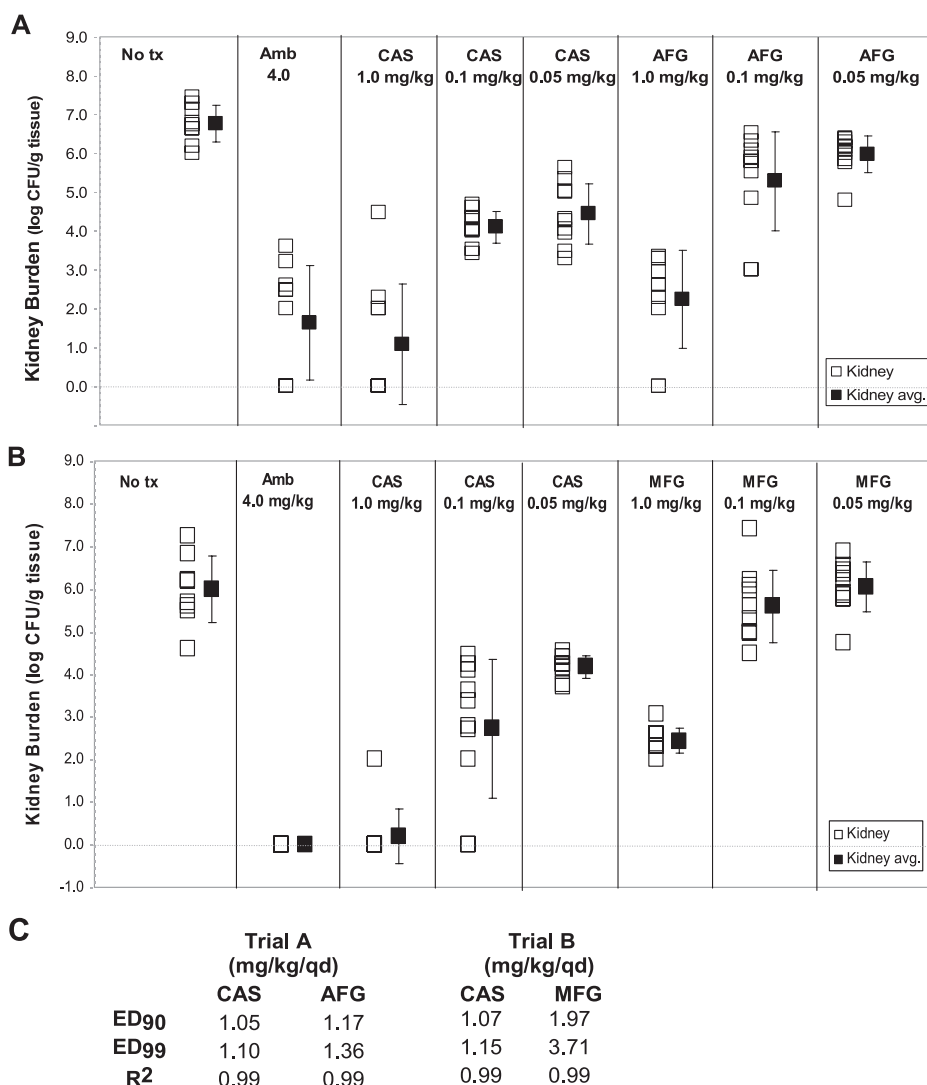

FIG. 2. Relative efficacies of caspofungin and micafungin in a murine candidiasis model. BALB/c mice (10 to 12 weeks; weight, 20 to 25 g) were infected by intravenous injection in the tail vein with 5×10^5 cells of *C. albicans* strain 36082. After 24 h, they were treated daily for 14 days at 0.05, 0.1, or 1 mg/kg/day with either caspofungin (CAS) and anidulafungin (AFG) (A) or CAS and micafungin (MFG) (B). The mice were evaluated for fungal kidney burdens, expressed as log CFU. Negative sham (no drug [No tx control]) and positive ambisome (Amb) (4 mg/kg/day) controls are indicated. Panel C shows 90 and 99% effective doses (ED_{90} and ED_{99}, respectively) obtained for the two drug trials (panels A and B). qd, once a day.

TABLE 3. IC$_{50}$ for glucan synthase from *Candida* and *Aspergillus*

Strain	EC$_{50}$ of drug for glucan synthase (ng/ml)[a]											
	Caspofungin				Micafungin				Anidulafungin			
	0	10	20	50	0	10	20	50	0	10	20	50
A. fumigatus												
R21	1.6	2.7	3.6	10.3	3.7	6.2	10.3	23.2	0.75	1.9	3.1	11.9
MF5668	1.1	1.0	3.2	4.8	2.2	3.2	3.3	7.6	0.29	5.5	6.9	28.1
ATCC 13073	2.0	2.8	5.9	9.4	2.0	4.7	7.5	9.8	13.3	21.4	77.4	
C. albicans												
ATCC 90028	0.3	1.3	2.1	6.3	18.0	28.4	50.1	90.2	1.00	4.72	18.6	24.7
ATCC 36082	0.9	2.8	5.3	8.7	10.8	23.7	31.4	57.0	18.5	24.0	71.4	
SC5314	14.7	27.0	36.9	80.5	26.9	32.6	50.6	89.7	17.1	15.3	74.7	89.0

[a] EC$_{50}$s (50% effective concentrations) of each drug are sorted by percentage of serum used (given below drug name).

fungal burdens (99% effective dose) in a candidiasis model (Fig. 2).

The effect of serum on echinocandin efficacy reflects known serum binding properties of echinocandin drugs. Micafungin is 99% protein bound, primarily to albumin and alpha-1-acid glycoprotein (1). Caspofungin is 96.5% protein bound, primarily to albumin (14, 15), and protein-bound drug can be recovered from target tissues (4). Anidulafungin was reported to be >84% protein bound (15), but it may be >98% bound (D. Sheehan, personal communication). It is not clear whether echinocandin drugs are active in the presence of bound serum protein or if a small proportion of free biologically active drug is available in the presence of serum.

In summary, human serum shifts the apparent antifungal potencies of echinocandin drugs in in vitro growth assays with diverse *Candida* spp. and *Aspergillus* spp., yielding nearly equivalent MICs or MECs, respectively. This behavior effectively negates any in vitro differences in potency between the drugs observed in standard testing medium. The primary effect of serum binding to this class of drugs appears to be a concomitant decrease in inhibition of glucan synthase.

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