

Paradoxical Effect of Caspofungin: Reduced Activity against *Candida albicans* at High Drug Concentrations

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Resistance problems with caspofungin, an echinocandin inhibitor of fungal cell wall glucan synthesis, have been rare. We noted paradoxical turbid growth of *Candida albicans* isolates in broth in some high (supra-MIC) concentrations. Among isolates submitted for susceptibility testing and screened at drug concentrations up to 12.5 µg/ml, the frequency was 16%. Analysis of the turbid growth indicated slowing of growth in the presence of drug but with numbers of CFU up to 72% those of drug-free controls. Clearing of growth again by the highest drug concentrations produced a quadriphasic pattern in a tube dilution series. Cells growing at high drug concentrations were not resistant on retesting but showed the paradoxical effect of the parent. Among a selected series of isolates tested at concentrations up to 50 µg/ml, an additional 53% showed a “mini-paradoxical effect”: no turbid growth but incomplete killing at high concentrations (supra-minimum fungicidal concentration). These effects were reproducible; medium dependent in extent; noted in macro- and microdilution, in the presence or absence of serum, and on agar containing drug (but not when drug concentrations were not constant, as in agar diffusion); not seen with other echinocandins and less commonly in other *Candida* species; and not due to destruction of drug in tubes showing the effect. Cooperative enhancement of inhibition by a second drug could eradicate the effect. We postulate that high drug concentrations derepress or activate resistance mechanisms. The abilities of subpopulations to survive at high drug concentrations could have in vivo consequences.

Caspofungin is an antifungal drug of the echinocandin class. Agents of this class can be produced by several fungi. This newly introduced agent has as its mechanism of action non-competitive inhibition of synthesis of (1,3)-β-D-glucan, a principal constituent of fungal cell walls (4). It has been shown to be fungicidal in vitro against *Candida* species and efficacious in animal models of candidiasis and in clinical trials (4). Development of resistance is rare, even after prolonged treatment (4). We recently observed resistance to growth inhibition and killing among some clinical *Candida* isolates, but only at high concentrations of caspofungin, and here we report those observations in detail.

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MATERIALS AND METHODS

Isolates. Isolates were identified to species level by standard methods reported previously (12, 17), with molecular genotyping performed for a subset of the isolates (22).

Susceptibility testing. Broth macrodilution and microdilution testing (13) utilizing an 80% turbidity endpoint (6), determination of the minimum fungicidal concentration (MFC) (20), incorporation of drug into agar (2), assays of drug diffusion into agar containing organisms (16), and macrodilution checkerboard-type drug interaction studies (3) were performed as detailed elsewhere. The medium used, except where otherwise specified, was RPMI 1640 (13), which, along with yeast nitrogen broth (YNB) (7) and synthetic amino acid medium-

fungi (SAAMF) (9), was prepared as previously described. Caspofungin was purchased from the Santa Clara Valley Medical Center pharmacy as the product for clinical use, and micafungin and anidulafungin powders were provided by Fujisawa Healthcare (Deerfield, Ill.) and Eli Lilly & Co. (Indianapolis, Ind.), respectively. With several isolates, caspofungin powder provided by Merck Research Laboratories (West Point, Pa.) gave results indistinguishable from those with the clinical preparation.

RESULTS

Pattern of paradoxical effect and frequency of effect among unselected clinical isolates. The pattern of the paradoxical effect is shown in the prototype clinical isolate, 03-178 (Table 1). The paradoxical effect was seen in 3 other unselected isolates submitted for testing but not in 21 others, giving an estimated frequency of 16% (it is recognized that among isolates submitted for susceptibility testing, isolates from suspected or defined therapeutic failures, or from problematic epidemiologic settings, will likely be overrepresented). The caspofungin MICs for all of the isolates were ≤0.39 µg/ml.

Three (18%) additional isolates (95-68, 95-142, and 98-8) from a second group of 17 isolates selected from another study (22) showed the effect and were restudied five times each; each time, the effect was reproduced. They were then also tested in a broth microdilution system (13), where the same paradoxical effect was also seen. One isolate was tested in 10% serum, and the paradoxical effect was seen again, although both the MIC and the range of the paradoxical effect were shifted up to tubes fourfold higher in the dilution series compared to medium without serum. When tested in medium without serum at room temperature (22 to 25°C), control growth required three additional days to reach 4+ turbidity, and at that time, the MIC was

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TABLE 1. Paradoxical caspofungin effect

Isolate (method)	Growth at caspofungin concn ($\mu\text{g/ml}$) of:											
	0 ^a	0.012	0.025	0.05	0.09	0.19	0.39	0.78	1.56	3.13	6.25	12.5
03-178 (growth ^b)	4+	ND ^d	ND	ND	ND	ND	0	0	Trace	1+	2+	Trace
95-68 (growth ^b)	4+	3+	2+	1+	Trace	0	0	0	0	2+	2+	2+
95-68 (MFC plate ^c)	Confluent	ND	ND	ND	17	0	0	1	30	TN ^e	TN	TN

^a Control.

^b Growth relative to 4+ control, where 3+ is less than control to $\approx 80\%$ turbidity compared to control, 2+ is ≈ 50 to 80% , 1+ is 50 to 25%, trace is >0 to 25%, and 0 is a clear tube. Visual turbidity readings were confirmed by spectrophotometer (10). The standard range for caspofungin susceptibility testing for clinical isolates is 0.39 to 12.5 $\mu\text{g/ml}$, as stated in the text.

^c Colonies enumerated on each plate are shown. Each colony represents 1.3 to 4% of the CFU in the original inoculum [microscopically enumerated inoculum, 10^3 yeasts/ml; acceptable variation in CFU (0.5 to 1.5) $\times 10^3$ /ml] (28).

^d ND, not done.

^e TN, too numerous to count.

50 $\mu\text{g/ml}$ and the paradoxical effect could not be studied. All three isolates were of genotype A (12), but genotype A is the dominant type in this collection (22).

Demonstration of paradoxical effect on killing and definition of the range of inhibition. In subsequent studies, the lower range of drug dilutions was expanded to define the range of the effect. A representative study with one of the isolates from the preceding group is shown in Table 1 (isolate 95-68, growth). There were always three to six clear tubes between the two ranges of growth (three isolates, each tested five times). These studies also define the MIC below the ≤ 0.39 - $\mu\text{g/ml}$ cutoff used to define a susceptible isolate in clinical testing. The MICs for all three of the isolates with the paradoxical effect were 0.025 to 0.09 $\mu\text{g/ml}$ on repeated testing, and the isolates would be considered highly susceptible by routine in vitro testing of clinical isolates or in prior publications on caspofungin activity in vitro (4).

In these studies, MFCs were determined in order to ensure that the turbidity noted at the highest concentrations represented organisms that were viable at the time turbidity in the tubes was scored. A typical result is shown in Table 1 (isolate 95-68, MFC plate). In every experiment, and in all those described below, the paradoxical effect on turbid growth was accompanied by the same effect on killing. One may note in Table 1 the survival of a few colonies at a concentration just below that which fails to inhibit visible growth. Further definition of the amount of inhibition at drug concentrations that do, in contrast, permit turbid growth is addressed below.

The effect of higher concentrations of caspofungin: the mini-paradoxical effect. The studies described thus far have defined the paradoxical effect at drug concentrations up to 12.5 $\mu\text{g/ml}$. That range was derived largely from clinical susceptibility testing, since higher concentrations of the drug in blood are not achievable with present doses (4) and thus were thought to be irrelevant. Although the availability and expense of drug supplies also affect the range of concentrations that can be routinely studied, it was of interest to see, with selected isolates, whether the paradoxical effect could be demonstrated in isolates that did not otherwise show it by expanding the range.

In the second group of isolates, the 14 that did not demonstrate the paradoxical effect were restudied at drug concentrations up to 50 $\mu\text{g/ml}$. One isolate showed the paradoxical effect, but only with trace growth and only at 25 $\mu\text{g/ml}$. In contrast, six isolates did not show the effect.

With the remaining seven isolates, another phenomenon was

seen. Although clear tubes were noted at up to 50 μg of caspofungin/ml, from tubes at 12.5 and/or 25 $\mu\text{g/ml}$, subcultures revealed 4 to 27 colonies/MFC plate. It should be noted that these seven isolates did not show (i) either turbidity in any tubes or colonies on subculture at concentrations of 0.025 to 6.25 or 12.5 $\mu\text{g/ml}$ or (ii) either turbidity or growth on subculture at 50 $\mu\text{g/ml}$. The former finding describes a subthreshold version of the paradoxical effect (called the mini-paradoxical effect), in which apparently the survival of only a small number of cells becomes possible in the presence of a high concentration of drug. This finding suggests that, whatever the mechanism of resistance at high drug concentrations, it may be overcome by even higher concentrations. This observation is supported by another—the appearance, in a series of tubes with doubling drug dilutions, of turbidity in tubes at increasing concentrations, then a further increase in turbidity, and finally a decline in turbidity as the drug concentration further ascends (e.g., Table 1, isolate 03-178, growth). The patterns just described indicate that the paradoxical effect is quadriphasic: growth below the MIC; inhibition above the MIC (for three to six tubes in the drug series); then, in the isolates showing the effect, release of inhibition at higher concentrations; and finally, inhibition again at the highest drug concentrations.

Another isolate, used in many animal model studies (21), that was studied concurrently showed only the mini-paradoxical effect (at 6.25 and 12.5 $\mu\text{g/ml}$), with colonies appearing only after 5 days of incubation. Thus, in this series of experiments, of 15 isolates that did not demonstrate the paradoxical effect, 8 (53%) showed the mini-paradoxical effect.

The preceding sets of results raise the issue of whether, if drug concentrations are elevated high enough, a paradoxical or mini-paradoxical effect might be seen in all *Candida albicans* isolates. However, studies at up to 400 $\mu\text{g/ml}$ with two of the isolates that failed to show these effects at concentrations up to 50 $\mu\text{g/ml}$ still failed to demonstrate turbidity or positive subcultures.

Magnitude of growth permitted at high concentrations. Isolates 95-68, 95-142, and 98-8 were studied to quantitate the amount of growth, corresponding to turbid tubes, permitted at high drug concentrations. Tubes of isolate 95-68 were prepared by the standard method (13) (10 each at 0 and 12.5 μg of caspofungin/ml for the usual 48 h), and cell pellets were prepared by centrifugation. The pellets in the 0- $\mu\text{g/ml}$ tube were 0.01 ml each, and the pellets in the 12.5- $\mu\text{g/ml}$ tube were 0.008

ml each (means), i.e., 80% of the volume in the absence of drug.

All three isolates were also inoculated into tubes with 0 and 12.5 μg of drug/ml, and at 48 h, subcultures were made from the pairs of turbid tubes. The subcultured fluid was serially 10-fold diluted, and the dilutions were plated in triplicate (20). CFU were enumerated on plates with 10 to 100 colonies. Growth in the absence of drug revealed $10^{6.7}$, $10^{6.8}$, and $10^{6.9}$ CFU/ml (means), and in 12.5 μg /ml, $10^{5.7}$, $10^{2.6}$, and $10^{4.5}$ CFU/ml. However, the accuracy of the CFU determination was questioned, because it was noted that there was clumping of the growth in drug. Microscopic observation of the growth in drug revealed budding yeast typical of *C. albicans* growth but failure of daughter cell separation in the presence of drug compared to growth in the absence of drug. The fact that there was still a drug effect on growth at the high concentration was also noted in another observation; subcultures for MFC determination from tubes showing the paradoxical effect demonstrated tiny colonies, particularly in the initial 10-fold dilutions, compared to colonies from subcultures of the drug-free growth. To test whether this observation was related to a new property of the cells growing at high drug concentrations rather than a continuing effect of the drug carried over from the tube to the subculture, an experiment was performed in which subcultures from tubes with 12.5 μg of drug/ml were pelleted by centrifugation, washed with fresh RPMI 1640 medium, recentrifuged, and resuspended in fresh medium, as opposed to no treatment of the subculture. The washed cells produced much larger colonies, corroborating a drug effect, as suggested by the clumping and the failure of bud separation in broth noted earlier.

Because of possible inaccuracies in quantitating the growth permitted, a population analysis was performed in another experiment by subculture of drug-free growth to RPMI agar plates (100 cells/plate, in triplicate, per isolate) containing 0 or 12.5 μg of caspofungin/ml. This method demonstrated that survival was 72, <1, and 38% (the percentage of CFU on the drug-containing plates relative to the drug-free plates) for the three isolates; this is the same rank order as quantitated growth of the three isolates from liquid medium with 12.5 μg of caspofungin/ml in the preceding experiment. The data for isolate 95-68 were also similar for percent volume of packed cells growing in drug compared to in the absence of drug and for this population analysis result.

Phenotype of survivors at high concentrations. To determine if there was possible selection of a resistant subpopulation in the growth permitted at high drug concentrations, colonies were selected from the plates subcultured from tubes with turbid growth at 12.5 μg of drug/ml (isolates 95-142, 95-68, and 98-8). Five colonies were selected for each isolate, and each was regrown in the absence of drug to produce an inoculum for susceptibility testing. In 15 of 15 instances, the progeny did not demonstrate total resistance but reproduced the paradoxical effect in the same fashion as the parent, which was tested concurrently.

Integrity of drug at high concentrations. To assess whether the mechanism of resistance at high drug concentrations might be due to alteration by cells of the drug in tubes, the following experiment was performed. Isolate 95-68 was grown in the presence of 12.5 μg of drug/ml for the usual incubation time

(13), and an identical tube, but without fungal cells, was incubated for the same period. At the end of the incubation, the tube with cells was centrifuged to pellet the cells, and the supernatant was aspirated for bioassay in triplicate, along with the contents of the uninoculated tube. The drug was present at 10.5 and 10.4 μg /ml, respectively, indicating no destruction of the drug by a subpopulation of the cells to explain the resistance noted.

Effects of medium and method. To assess the effect of the medium on the paradoxical effect, three media (RPMI 1640, the medium used in all studies described above; SAAMF; and YNB) were studied with each of three isolates demonstrating the paradoxical effect at drug concentrations of 0.39 to 50 μg /ml. The paradoxical effect was seen in all nine instances. The effect was modestly less pronounced in YNB and more pronounced in SAAMF compared to RPMI. For example, isolates showed only turbid growth at ≥ 12.5 μg of drug/ml in YNB, whereas turbidity occurred in tubes with ≥ 3.1 μg of drug/ml in SAAMF, and turbidity in RPMI was seen, on average, beginning one twofold drug dilution higher than in SAAMF. Subcultures from SAAMF tubes with paradoxical turbidity also generated, in most instances, more CFU at the same drug concentrations than with RPMI. It should also be noted that MICs of caspofungin in SAAMF were generally four twofold dilutions higher than those described in RPMI (0.78 to 1.56 μg /ml in SAAMF), and thus, the zone of clear tubes between sub-MIC and paradoxical-effect concentrations also shrank from three to six tubes to one or two tubes in SAAMF. Both RPMI and SAAMF are well buffered to physiologic pH, suggesting that the medium differences are not due solely to the pH.

We have already indicated that the paradoxical effect can be seen in broth dilution and on agar plates containing caspofungin. Isolates were also incorporated into agar to form lawns of growth as in a bioassay (16), and twofold dilutions of caspofungin (0.39 to 25 μg /ml) in distilled water were placed in wells cut in the agar. With all three isolates demonstrating the paradoxical effect in broth dilution that were studied, clear zones were produced with 0.39 or 0.78 to 25 μg of drug/ml. Interestingly, each twofold-higher drug concentration produced a larger zone than the concentration below it, and no colonies were noted growing within the zones.

Specificity of the paradoxical effect among echinocandins. Forty-six clinical isolates of *C. albicans* from micafungin clinical trials and 14 isolates from the present study (which did not show the caspofungin paradoxical effect) were tested for susceptibility to micafungin at concentrations from 0.06 to 16 μg /ml; the paradoxical effect was not noted (all MICs were ≤ 2 μg /ml, and 98% were ≤ 0.25 μg /ml; 58 isolates were tested for MFC, and all were ≤ 2 μg /ml; no mini-paradoxical effect seen in these isolates either). To confirm this negative result, three isolates demonstrating the paradoxical effect with caspofungin were tested with micafungin at 0.125 to 128 μg /ml, with anidulafungin at 0.39 to 50 μg /ml, and with caspofungin; no paradoxical effect or mini-paradoxical effect was seen with the first two drugs (MICs, ≤ 0.125 and ≤ 0.39 , respectively).

Effect of a second antifungal on paradoxical effect. The effect of the interaction of a second antifungal with caspofungin on the paradoxical effect was of interest. Fluconazole and caspofungin were therefore studied in standard checkerboards

with isolates 95-142, 95-68, and 98-8 (the last is susceptible to fluconazole; the others are resistant).

With isolate 98-8, the interaction seen in the caspofungin MIC range was synergy (fractional inhibitory concentration index, ≤ 0.38). The others had fractional inhibitory concentration indices of just slightly >0.5 . Of most interest, however, with 95-142 and 95-68 was the fact that the presence of fluconazole, including several concentrations below the fluconazole MIC, could abolish the paradoxical effect of caspofungin at high concentrations. For example, whereas 1+ growth was noted for 95-68 with 6.25 μg of caspofungin/ml alone, with colonies too numerous to count on subculture for MFC determination, with 16 μg of fluconazole/ml also present (one-fourth the MIC), a clear tube resulted, and with 32 μg of fluconazole/ml, there was a clear tube with no colonies on subculture ($\geq 96\%$ killing). (With isolate 98-8, the paradoxical effect with caspofungin alone occurred in concentrations just above those in the drug combination tubes in the checkerboard, so this aspect with that strain could not be evaluated in this study.)

Trailing endpoint isolate. An isolate (03-202), one of several selected from a multicenter collaborative study of in vitro susceptibility testing of caspofungin that were believed to show a trailing endpoint up to high caspofungin concentrations, was kindly provided by Frank C. Odds. We found this isolate to be susceptible to caspofungin (MIC, 0.09 $\mu\text{g}/\text{ml}$) but to display a protracted paradoxical effect. At 0.19 to 0.39 $\mu\text{g}/\text{ml}$, clear tubes were noted, but from the 0.39- $\mu\text{g}/\text{ml}$ tube, many colonies were subcultured on the MFC plate. At 0.78 $\mu\text{g}/\text{ml}$, trace growth appeared, with the turbidity increasing in ascending drug concentrations to a maximum (2+) at 6.25 $\mu\text{g}/\text{ml}$ and then declining, so that at 25 to 50 $\mu\text{g}/\text{ml}$, clear tubes were produced with no colonies on subculture. With isolates such as this, if sufficiently low drug concentrations are not used in screening, the chance of defining a drug effect as prominent as the abolition of visible growth could be missed.

Candida species other than *C. albicans*. Caspofungin broth dilution testing was performed with 47 isolates of other *Candida* species (20 *C. glabrata*, 9 *C. parapsilosis*, 8 *C. tropicalis*, 3 *C. kefyr*, 3 *C. krusei*, 2 *C. lusitaniae*, 1 *C. zeylanoides*, and 1 *C. intermedia*), and the paradoxical effect was seen only in 1 *C. tropicalis* and 2 *C. parapsilosis* isolates. With one of the latter, none of the tubes in the zone of clear tubes between the MIC and paradoxical growth achieved the $\geq 96\%$ killing cutoff, but there was also an increase in colonies on the MFC plates at the concentrations where turbid growth occurred. MFC testing was performed with 14 of the isolates that did not show the paradoxical effect, and the mini-paradoxical effect was not seen, although with 3 *C. parapsilosis* isolates, the MFC was off the scale (>25 $\mu\text{g}/\text{ml}$), and no mini-paradoxical effect could have been seen. Micafungin testing was performed with 51 isolates (18 *C. glabrata*, 15 *C. parapsilosis*, 11 *C. krusei*, 2 *C. tropicalis*, 2 *C. lusitaniae*, 1 *C. rugosa*, 1 *C. kefyr*, and 1 *C. intermedia*), and the paradoxical effect was not seen. MFC testing was performed with 47 of these isolates, and the mini-paradoxical effect was not seen, although with 1 isolate the MFC was higher than the highest dilution tested, and no mini-paradoxical effect could have been seen.

DISCUSSION

We have demonstrated a paradoxical effect, in a minority of *C. albicans* isolates, in which growth occurs at concentrations of caspofungin well above the MIC and MFC. This phenomenon appears to be specific to caspofungin among echinocandins, is affected in degree by the medium used for the susceptibility assay, and appears to be less common with other *Candida* species. A possibly similar phenomenon for caspofungin was suggested, but not explored, in prior observations (1, 15). The surviving cells at high concentrations appear to be still subject to some drug effect, showing evidence of slowed growth in the presence of the drug. Medium dependence with classical MIC results has also been noted previously (14). The concentrations of caspofungin in vitro at which we have demonstrated the phenomenon are well within the range achieved in patients with the present dosing: the mean peak serum concentrations are 12.1 $\mu\text{g}/\text{ml}$ after a single 70-mg dose, and concentrations in some tissues appear to greatly exceed that in serum (4).

We have shown that this growth is not due to selection of a resistant subpopulation. If it were due to a resistant subpopulation, one would expect to see the paradoxical growth at all concentrations above the MIC and possibly preferentially at lower concentrations, not preferentially at higher concentrations. Moreover, we show that progeny of the growth at high concentrations retain the phenotype of the parent.

The effect can occur to the point of producing turbid growth, but a similar propensity, to a lesser degree (i.e., in a smaller fraction of the cell population), so that only incomplete killing occurs at higher concentrations (the mini-paradoxical effect), appears to occur more commonly among isolates. We have shown that the paradoxical effect occurs in broth dilution and agar dilution (where the drug is incorporated into agar) but is not seen when the drug is diffused from wells into agar supporting fungal growth. Methodological differences in results are reminiscent of reports of "resistance" to caspofungin demonstrable by some methods (agar dilution) but not others (broth dilution) (18). We note that the situation where the drug diffuses into agar (which, like broth or agar dilution, can also be used to define the susceptibility of an isolate [the MIC; defined in reference 9]) is, in contrast to broth or agar dilution, the only method where the isolate does not experience a constant drug concentration during the incubation period. In the first method, the isolate experiences a changing concentration of drug during its growth as the drug diffuses radially into the agar. The method dependence we observed is consistent with the hypothesis that the paradoxical effect is due to derepression (or activation) of a resistance mechanism induced by constant high drug concentrations. The hypothesized derepression or activation occurs rapidly, in 48 h of a single pass in the presence of drug. The ability to derepress would provide a survival advantage in the presence of drug to some strains or their subpopulations. This survival advantage could occur in nature, since the class of echinocandins represents various synthetic modifications of a natural product. The appearance of this phenomenon is not a consequence of the introduction of the echinocandins into clinical use, since most strains in which we demonstrated the paradoxical effect were isolated prior to such introduction. The putative derepressed resistance mechanism could be related to an intrinsic change in the target

glucan synthase complex, access to it, compensatory upregulation of synthesis of another wall component, etc.

An analogous phenomenon has been described in some bacteria in the presence of penicillin (5). Penicillin, analogous to caspofungin's effect on fungi, acts by blocking microbial cell wall synthesis. A similar paradoxical effect with semisynthetic penicillins has been attributed to derepression in bacteria of a resistance gene by high drug concentrations (10). An alternative explanation for the paradoxical effect could be an alteration in the physical state of the drug (e.g., aggregation) at high concentrations, but such a hypothesis would not easily explain the presence of the effect in some *Candida* isolates but its absence in others, the suppression at very high concentrations, or the persistence of most of the drug in active form at the end of incubation, and clinically, infusion of high concentrations of the intravenous preparations appears not to be attended by drug inactivation problems.

The most extreme example of the paradoxical effect was the highly selected isolate, 03-202, which may have the cells most sensitive to derepression, since breakthrough growth occurred in concentrations as low as 0.39 $\mu\text{g/ml}$ (even in RPMI). Identifying isolates with trailing endpoints may be an easy way to find, in large-scale screens of susceptibility, isolates with a propensity for paradoxical growth at concentrations just above the MIC. One of the four "random" isolates demonstrating the paradoxical effect uncovered in routine susceptibility testing also had this extensive phenotype.

The paradoxical effect appears to have no analogy to the phenomenon of heteroresistance described (11) in some *C. albicans* strains with azoles, as there is no evidence that strains showing the paradoxical effect have resistant clones at low concentrations (but above the MIC and MFC) of caspofungin (all cells are inhibited and killed). The paradoxical effect requires high concentrations of drug, which in a single exposure (as opposed to repeated or prolonged exposure to drug) rapidly induces resistance, and in a high proportion of cells; the drug exposure selects only for transient resistance (the progeny revert in a single pass to the same phenotype as the parent), and colonies from strains showing the paradoxical effect do not appear within a zone resulting from the diffusion of drug from wells.

The hypothesized derepressed resistance mechanism does not involve destruction of the drug by a fraction of the fungal cells. Whether the mechanism could be related to the ability of some *C. albicans* isolates to switch phenotypes (19), a potential survival advantage in some situations, is the subject of study, as are any possible relationship with azole resistance mechanisms; assessment of possible mutations in resistance-associated regions of *FKSI*, a gene encoding a key protein in glucan synthase; and measurement of glucan synthase enzyme activity in the cells exposed to the drug.

The possible in vivo significance of the paradoxical effect is also under study in an animal model. An effect in which up to 72% of a fungal population can survive in vitro in the presence of high drug concentrations could conceivably have in vivo consequences. Correlation of the effect in isolates in association with caspofungin clinical treatment successes versus failures would be of interest, if such isolates were available. If there is in vivo significance for the paradoxical effect, it may prove useful to know that a second drug can act cooperatively,

in the presence of caspofungin, to clear the paradoxical growth as shown, although an in vivo demonstration of the cooperative effect would be needed (8).

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