Effect of Candida Morphology on Amphotericin B Susceptibility

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We showed that brief exposures to amphotericin B (AmB) inhibited the induction of new Candida germ tubes and the lengthening of partially induced germ tubes. Blastocidin with germ tubes were more susceptible to AmB killing, and this varied directly with the induction period and the AmB exposure period. AmB did not preferentially affect germ tube adherence to fibrin matrices.

We recently showed that sublethal concentrations of amphotericin B (AmB) inhibited germ tube formation in Candida albicans cultures (7). Niimi et al. reported that germ tube-forming cells of C. albicans are more susceptible to clotrimazole-induced killing than are yeast cells (6), and Borgers and co-workers found that pseudomycelium formation was very sensitive to dilute concentrations of imidazoles (1). These studies suggest that the biochemical and morphological changes associated with germ tube formation produce increased susceptibility to antifungal agents.

Germ tube formation. In earlier studies with a clinical isolate of C. albicans (11), we observed that AmB inhibited the induction of germ tubes in serum (7). In the experiments described here, we measured the effects of brief exposure to low concentrations of AmB (0.1 μg/ml) on germ tube development and lengthening in partially induced cultures. Blastocidin were incubated in 10% newborn calf serum in phosphate-buffered saline (NBCS; GIBCO Laboratories, Grand Island, N.Y.) for 45 min to induce germ tubes, incubated with AmB for 15 min, washed free of AmB, and then reincubated in 10% NBCS for an additional 60 min. After 45 min, 34.2 ± 9.9% (standard error) of blastocidin had germ tubes identified by light microscope criteria (9), blastocidin had more germ tubes per cell than did control blastocidin (Table 1).

Germ tube killing. In pilot experiments, we tested various AmB concentrations on C. albicans cultures which had been induced for 60 min at either 37 or 0°C in 10% NBCS. The results demonstrated that C. albicans with germ tubes (i.e., 37°C cultures) were more susceptible to AmB than control cultures incubated at 0°C (data not shown). On the basis of these results, we used AmB at concentrations of 0.25 μg/ml in the next three series of experiments. Blastocidinal suspensions were incubated in 10% NBCS for 0 to 120 min and then exposed to AmB for 60 min. Longer induction periods increased susceptibility to AmB killing, and the percent survival decreased from 97 ± 3% after 0 min of induction to 84 ± 7% after 30 min, 57 ± 13% after 60 min, and 13 ± 4% after 120 min (n = 6 for each time point). In a second series of experiments, germ tubes induced for 60 min were incubated with AmB for 0 to 120 min. Longer exposures produced greater killing, and AmB had a more pronounced effect on Candida cells with germ tubes (Fig. 1). Finally, germ tubes were induced in 10% NBCS for 60 min and then incubated with AmB for 60 min. Samples of these mixtures were supplemented with either KCl (85 mM) or MgCl2 (45 mM) during AmB exposure (3). Neither cation significantly prevented killing by AmB (data not shown; n = 6).

Candida adherence to fibrin matrices. AmB strongly inhibits the adherence of Candida blastocidin to fibrin matrices (7). In the present experiments, we compared the effect of AmB on germ tubes and on blastocidinal adherence to matrices prepared with thrombin-clotted fresh frozen plasma (4, 5). Control untreated blastocidin with germ tubes tended to adhere to fibrin better than did control blastocidin (56 ± 20 CFU/12 cm2 at 102 CFU input versus 20 ± 4 CFU/12 cm2 at 102 CFU input), but this difference did not reach statistical significance (by the paired t test). At low

<table>
<thead>
<tr>
<th>TABLE 1.</th>
<th>Germ tube length and numbera</th>
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<tr>
<td>Culture</td>
<td>No. of yeasts measured</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>164</td>
</tr>
<tr>
<td>AmB treated</td>
<td>155</td>
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a Cover slips with adherent C. albicans cells were fixed with 0.1 M phosphate-buffered 3% glutaraldehyde, postfixed with 0.1 M phosphate-buffered 2% OsO4 dehydrated in ethanol, mounted in toto on aluminum planchets, and sputtered with approximately 400 Å (40 nm) of gold/palladium. Specimens were examined at 20 kV, 0° tilt, and at ×600 magnification in an AMR 1000A scanning electron microscope with 1-μm-diameter latex reference beads. Germ tube lengths were measured with a cursor interfaced to a Zeiss Videoplan image analysis system.

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concentrations of AmB, blastoconidial adherence was more
sensitive to AmB than was germ tube adherence (Fig. 2).

In these experiments, low concentrations of AmB prefer-
entially affected Candida germ tubes. The explanation(s)
for this effect is uncertain but might include the following
considerations. Firstly, cell wall synthesis and minor
changes in membrane permeability during germ tube forma-
tion might allow increased AmB uptake, and this produces
the increased killing observed in germ tube cultures.
Secondly, during germ tube formation certain synthetic
activities are concentrated at restricted regions of the cell (10). It

is possible that AmB causes cell death by localizing to these
regions and preferentially inhibiting the formation of new
cell wall. Rast and Bartnicki-Garcia demonstrated that AmB
inhibits chitin synthesis at concentrations around 1 µg/ml (8).
This formulation would not require an increase in total drug
uptake and would be supported by the observation that germ
tube lengthening is inhibited by AmB but that new second
germ tubes can develop. Finally, AmB may have unknown
effects on critical membrane functions, and these effects may
become physiologically important during periods of in-
creased metabolic activity. The mechanism of action of
AmB at low concentrations is usually attributed to changes
in permeability which allow the release of critical ions and
nutrients (3). However, we could not prevent the loss of
viability by the addition of either Mg²⁺ or K⁺ to the germ
tube cultures, and the AmB incubation was performed in
fresh tryptic soy broth. At higher concentrations, AmB
causes lipid peroxidation in erythrocytes (2). Oxidative
injury probably occurs at lower concentrations and could
damage critical enzymes. This formulation suggests that
permeability measurements do not reflect AmB killing
events.

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