Comparison of Antifungal Activity of Amphotericin B Deoxycholate Suspension with That of Amphotericin B Cholesteryl Sulfate Colloidal Dispersion

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Amphotericin B (AmB) has been a useful antifungal agent for about 30 years and is the standard against which new drugs are usually measured. However, its well-known toxicities (7) have limited its clinical application. In its present clinical formulation, it is a micellar suspension with sodium deoxycholate (amphotericin B deoxycholate suspension [ABDS]; Fungizone; Bristol-Myers Squibb, Princeton, N.J.). Enveloping AmB in lamellar lipid vesicles (liposomes) has been a promising method of reducing toxicity and altering tissue distribution (11); however, such formulations are not easy to prepare or standardize. Lipid complexing of AmB is an attractive alternative for accomplishing the same goals, permitting a preparation that is uniform and reproducible. One such preparation (2) uses cholesteryl sulfate to form a stable colloidal dispersion with AmB (AmB colloidal dispersion [ABCD]; Liposome Technology, Inc., Menlo Park, Calif.). Because previous studies of liposomal AmB have indicated alteration of antifungal potency by the lipid component (5), it was of interest to assess the effect of lipid complexing on antifungal potency. ABCD was compared with ABDS in vitro against 41 isolates of 15 different fungal species, both primary pathogens and opportunistic fungi.

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All isolates had been cultured from patients with active disease. The media and methods used for testing each organism were those which had previously been shown in our laboratory to give satisfactory control growth and sharp endpoints for drug susceptibility testing (3). Appropriate biohazard class facilities were used. Each of the broth media used for MIC testing is a completely defined synthetic medium. No single medium will produce optimal control growth for all 15 species tested. However, it was shown in earlier studies that if one medium was chosen to test many species, the endpoints determined by using that medium would not be significantly different from those found using media that maximize growth of the organisms being tested (3). Details of methodology are further specified below and in Table 1. Table 1 details media and methods used to test each of the species of fungus listed. Three different completely defined synthetic media were used. The synthetic amino acid medium (4), modified as indicated in Table 1, and modified McVeigh-Morton medium (9) were prepared as described elsewhere. The reagent suppliers were Gibco Laboratories, Grand Island, N.Y.; Difco Laboratories, Detroit, Mich.; Becton Dickinson, Irvine, Calif.; and BBL Microbiology Systems, Cockeysville, Md. The fungal isolates were stored under carbon dioxide at 25°C or on slants at 4°C. To prepare the inoculum, growth was harvested from subcultures on appropriate media. Yeasts were incubated at 35°C in ambient air on a gyratory shaker (140 rpm) to produce a broth culture in growth phase. Optical densities of Candida spp. and Cryptococcus neoformans were measured spectrophotometrically; other yeasts were enumerated in a hemacytometer. Mycelial fungi were harvested from solid media and enumerated. All fungi were diluted in the appropriate broth medium and drug dilution so that the final inoculum was 10,000 cells per ml.

ABDS was supplied as a lyophilized powder containing sodium deoxycholate and buffered with sodium phosphate. It was reconstituted with sterile water, stored frozen, and protected from light. ABCD was supplied as a lyophilized powder, reconstituted in sterile water, stored at 4°C protected from light, and used within 1 week. A twofold dilution series of each drug was prepared in medium. Final concentrations in tubes with the inocula ranged from 16 to 0.125 μg/ml.

Plastic tubes with loose flip caps were used for the MIC determination. The broth volume in each tube was 2 ml. Each dilution series included a tube with medium but no drug. A dilution of the inoculum was placed on solid medium to verify absence of contamination, inoculum quantitation,
TABLE 1. Details of antifungal MIC and MFC determinations for various isolates

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum preparation</th>
<th>MIC</th>
<th>MFC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Form tested</td>
<td>Medium</td>
<td>Incubation</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>Y</td>
<td>YNB</td>
<td>24 h</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>Y</td>
<td>SAAMF</td>
<td>48 h</td>
</tr>
<tr>
<td>Histoplasma capsulatum</td>
<td>Y</td>
<td>SAAMF</td>
<td>48 h</td>
</tr>
<tr>
<td>Blastomyces dermatitidis</td>
<td>Y</td>
<td>BAP</td>
<td>72 h</td>
</tr>
<tr>
<td>Paracoccidioides brasiliensis</td>
<td>Y</td>
<td>MMVM</td>
<td>7 days</td>
</tr>
<tr>
<td>Sporothrix schenckii</td>
<td>Y</td>
<td>GYE</td>
<td>4 wk</td>
</tr>
<tr>
<td>Coccioidiose immitis</td>
<td>M</td>
<td>PDA</td>
<td>7 days</td>
</tr>
<tr>
<td>Rhizopus sp.</td>
<td>M</td>
<td>PDA</td>
<td>7 days</td>
</tr>
</tbody>
</table>

* Media used: YNB, yeast nitrogen broth (Difco) with 0.5% glucose, pH 5.4; SAAMF, synthetic amino acid medium for fungi, pH 7.4, with 0.165 M MOPS (morpholinepropanesulfonic acid) without Tris, cysteine, or cystine; MMVM, modified McVeigh-Morton medium, pH 7.0; BAP, blood agar plates (Becton-Dickinson); SDA, Sabouraud dextrose agar (Difco); FDA, potato dextrose broth (Difco) with 3% agar (BBL); GYE, 2% glucose–1% yeast extract (Difco) with 2% agar (BBL); BHI, brain heart infusion agar (BBL) supplemented with 10% horse serum (GIBCO) and 15% P. brasiliensis culture filtrate. Other abbreviations: Y, yeast cells; M, mycelia; OD100, spectrophotometric determination of optical density at 540 nm; counted, counted in a hemacytometer chamber.

and viability. The tubes were incubated until sufficient growth appeared in tubes containing medium without drug. The tubes were protected from light during incubation. Growth in tubes containing drug was compared with growth in tubes containing medium without drug. The MIC was considered the lowest concentration resulting in no evident growth.

To determine the minimum fungicidal concentration (MFC), plates containing appropriate solid media were divided into quadrants. From each dilution series, contents of the tubes without evident growth or with questionable trace growth and of the tube with no drug were vigorously mixed, and 0.04 ml was removed from each tube and placed on a quadrant. The plates were incubated until growth from tubes containing no drug was evident. The MFC was considered the lowest concentration producing one or no colonies on the plate. Tubes with questionable trace growth which produced one or no colonies in the MFC assay necessitated a rare reassessment of the MIC designation; such tubes, which met the criteria for fungicidal activity, were also considered to be at or below the MIC. A known ABDS-susceptible organism was assayed periodically during these experiments to confirm drug potency and assay reproducibility, and the results were always ≤1 twofold dilution different from that recorded previously in our laboratory.

The results are shown in Table 2. The range of MICs of ABDS was 0.25 to 4.0 μg/ml, and MICs of ABCD were ≤0.125 to >16 μg/ml. For 31 of 41 isolates, the ABCD MIC was ≤4 μg/ml. For 16 isolates, the ABCD MIC was lower than that of ABCD; for 16, the reverse was true; and for 9, the MICs were identical. In 15 of the 32 instances of differences in MICs, the differences were considered large (fourfold or more), and they appear nondemanding in that 12 of these 15, the ABDS MIC was lower. Notably, these 12 included 8 (of the 11) Aspergillus isolates.

The MFC ranges were 0.25 to >16 and ≤0.125 to >16 μg/ml for ABDS and ABCD, respectively. For 17 isolates, the ABDS MFC was lower than that of ABCD; for 17, the reverse was true; and for 7, the MFCs were identical. In 17 of the instances of discordance, the differences were large, and in 12 of these, the ABDS MFC was lower. Discordances were not reported with liposomal preparations and ABDS (10).

Organisms for which differences in susceptibility were
suggested were *Aspergillus* spp. and *Torulopsis glabrata*, for which only 2 or 11 and 0 of 2 instances, respectively, had an ABCD MIC or MFC lower than that of ABDS. The single isolates of *Sporothrix schenckii* and *Rhizopus* spp. tested had lower MICs and MFCs of ABDS. Subsequent studies, perhaps particularly if they used different in vitro methodologies, would be of interest to confirm whether fungi of these four genera are possibly more ABDS susceptible. On the other hand, for no isolates of *C. neoforms*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, or *Paracoccidioides brasiliensis* (five, three, two, and two isolates studied, respectively) was the ABDS MIC or MFC lower than that of ABCD. These yeast pathogens may prove consistently more susceptible to ABCD. The results with *Candida* and *Coccidioides* spp. were variable; with the latter, ABCD MICs were always lower and MFCs were higher. Although shaking of cultures has been reported to affect the in vitro activity of liposomal preparations (8), this could not explain the species differences seen in these studies (Tables 1 and 2).

Thus, ABCD complexing variably affects AmB in vitro activity compared with that of ABDS. Neither preparation was favored in terms of number of isolates with higher or lower results in either the MIC or MFC assays. In less than a third of comparisons are there large (fourfold or more) decreases in activity with ABCD complexing, and in approximately 10%, there is a large increase. AmB complexed in ABCD apparently is generally able to reach its fungal targets. Some trends between fungal species may have emerged in these studies. Since animals can tolerate larger doses of ABCD than of ABDS and since the organ distribution of the two preparations is different (1, 2, 6), the possible significance of these in vitro differences must be addressed in in vivo studies. This is a potentially important issue, since clinical trials with ABCD are beginning.

REFERENCES