Comparative In Vitro Antifungal Activity of Amphotericin B and Amphotericin B Methyl Ester

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Received for publication 26 August 1974

The in vitro antifungal activity of amphotericin B methyl ester (AME), a water-soluble derivative of amphotericin B, was compared to that of the parent compound against a variety of pathogenic and potentially pathogenic fungi. AME has a significant antifungal activity, but the activity of AME was slightly lower than that of amphotericin B. Among the yeast-like organisms, only the yeast cells of Sporothrix schenckii were more resistant than others to both antibiotics, with a minimal fungicidal concentration of 5 to 10 µg/ml. The yeast cells of other fungi were killed at concentrations of 1 µg or less of either antibiotic per ml. The filamentous forms of S. schenckii and Oidiodendron kalrai were more resistant than the filamentous forms of other dimorphic fungi to both drugs. The minimal fungicidal concentration for S. schenckii was 10 µg/ml and for O. kalrai, 50 µg/ml. The dermatophytes, phycomycetes, and dematacous and other potentially pathogenic fungi were inhibited fairly well by both drugs, but up to 50 µg/ml was required for fungicidal action. The water solubility and wide spectrum of antifungal activity of AME warrant evaluation of its chemotherapeutic activity against experimental fungal infections.

Amphotericin B, a polyene antibiotic produced by Streptomyces nodosus, is the most effective agent currently available for the treatment of many systemic mycoses. The drug is administered in a dispersed form (Fungizone), using sodium deoxycholate as the dispersing agent. The utility of amphotericin B, however, is seriously hampered by its nephrotoxicity and other side effects (2, 4, 10, 11), which in part may be associated with its insolubility in water and other body fluids. Previous attempts in the chemical alterations of the polyene antibiotic, with the hope of reducing the toxicity, have not been successful (2, 5). Chemical changes in the antibiotic, resulting in increased solubility or decreased toxicity, were usually associated with a decrease in antifungal activity.

Recently, Mechlinski and Schaffner (6) have prepared amphotericin B methyl ester (AME) hydrochloride, a water-soluble derivative of amphotericin B. AME when dissolved in water formed micelles, but the degree of dispersion was significantly higher than that of Fungizone (1). AME hydrochloride has been shown in experimental animals to be significantly less toxic than the parent compound (3).

The lower toxicity and higher solubility of AME and preliminary demonstration of its antifungal activity against Saccharomyces cerevisiae and Candida albicans (1) led us to a more comprehensive study of the antifungal activity of the new derivative. The study reported here describes the comparative in vitro antifungal activity of AME with that of amphotericin B against a number of pathogenic and potentially pathogenic fungi.

MATERIALS AND METHODS

Cultures. Sixty-one isolates of pathogenic and potentially pathogenic fungi used for susceptibility testing were obtained from our stock culture collection. These included: Aspergillus fumigatus, A. niger, Blastomyces dermatitidis, C. albicans, C. guilliermondii, C. pseudotropicalis, Cladosporium sp., Coccidioides immitis, Cryptococcus neoformans, Histoplasma capsulatum, H. duboisii, Microsporum canis, M. gyipseum, Mucor pusillus, Oidiodendron kalrai, Paracoccidioides brasiliensis, Philalophora compactum, P. dermatitidis, P. verrucosa, Rhizopus arrhizus, Sepedonium sp., Sporothrix schenckii, Syncephalastrum, Torulopsis glabrata, Trichophyton mentagrophytes, T. rubrum, and T. simii.

The yeast-phase cultures were maintained on modified brain heart infusion agar slants (Difco) supplemented with 1.0% glucose and 0.1% L-cysteine hydrochloride. Cultures were maintained at 4 C and transferred every 3 weeks. The mycelial-phase cultures were maintained on Sabouraud dextrose agar slants at 4 C and transferred every 3 months.

Media. The antibiotic susceptibility was per-
formed in a synthetic medium (pH 7.2) (8) with the following composition (grams per liter): K2HPO4, 2.5; NH4Cl, 0.5; (NH4)2SO4, 0.5; L-cysteine hydrochloride, 0.1; MgSO4·7H2O, 0.1; glucose, 5.0; glutamine, 0.1; and FeCl3, 0.001. The solid synthetic medium contained 1.5% agar (Difco). The medium was dis- 
Ppersioned in 100-ml quantities into 250-ml Erlenmeyer 
flasks and sterilized by autoclaving.

Preparation of inoculum. The yeast cells were grown on brain heart infusion agar slants at 37°C for 24 to 48 h depending on the organism. The growth was harvested with 10 ml of sterile saline, and 2 ml of this suspension was inoculated into flasks containing 50 
ml of the liquid synthetic medium. The flasks were inoculated at 37°C for 24 to 48 h on a gyrotory shaker (New Brunswick Scientific Co.) with a shaking speed 
of 150 rpm. The cultures were harvested, washed twice with cold sterile 0.9% 
saline, and centrifuged in a refrigerated 
Sorvall twice with cold sterile 0.9% 
saline solutions containing 10,000 to 
24 
suspension was inoculated into 
the 
mycelial-phase 
cultures 
were 
incubated 
for 24 to 48 h on a 
gyrotory shaker 
and 
refrigerated 
Sorvall 
twice with cold sterile 0.9% 
solutions 
containing 
10,000 to 
24 
suspension 
was 
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and 
refrigerated. 
The 
mycelial-phase 
cultures 
were 
incubated at 37°C for 
24 to 
48 h on 
a 
gyrotory 
shaker. 

Preparation of antibiotic dilutions. Amphotericin 
B and AME hydrochloride were dissolved in sterile 
dimethylsulfoxide and diluted with distilled water to 
to 
obtain solutions containing 10,000 to 1.0 
μg of the 
antibiotic per ml. Appropriate amounts of these 
solutions were dispensed into flasks containing 100 
ml of the 
liquid and solid synthetic media to obtain final 
concentrations of the drugs ranging from 50.0 to 0.01 
μg/ml. The liquid medium containing antibiotics 
was 
dispensed in 5-ml quantities into sterile test tubes, 
and 
the 
medium was 
poured into sterile petri 
dishes.

Susceptibility testing. Both tube and agar dilu-
tion techniques were used to determine the antibiotic 
susceptibility. The standardized cell suspension in 
0.05-ml quantities was inoculated with a calibrated 
loop into liquid and solid media containing varying 
amounts of the antibiotics. Controls included: un-
inoculated 
sterility control, drug- 
and 
solvent-free 
growth controls, and growth controls containing 1% 
of 
the 
solvent. All tests were performed in triplicate.

Inoculated 
tubes 
and 
plates 
were 
incubated at 30°C 
for 
24 to 48 h depending 
in the 
controls. The 
minimal time of incubation was 48 h. After determin-
ing the 
minimal inhibitory concentrations (MIC), 0.05 
ml of 
agar 
or 
broth was transferred from the test 
media 
showing 
no growth 
and 
from 
the 
first 
cultures 
in which growth was detectable to the synthetic 
agar 
plates. The 
plates 
were 
icubated at 30°C 
for 
72 h or 
until 
growth 
appeared 
on the 
plates 
iculated from 
test 
media 
containing 
visible 
growth.

The 
criteria 
used 
to 
determine 
the 
MIC 
and 
mini-
mal fungicidal concentrations (MFC) were adapted 
from 
those 
described 
by 
Shadomy (8). The lowest 
concentration 
of 
antibiotic 
which 
completely 
inhb-
**Table 1. In vitro susceptibility of yeast-like organisms to amphotericin B and AME by the agar dilution technique**

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Strains tested</th>
<th>Susceptibility to Amphotericin B</th>
<th>Susceptibility to AME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MIC (µg/ml)*</td>
<td>MFC (µg/ml)</td>
</tr>
<tr>
<td><em>H. capsulatum</em></td>
<td>4</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td><em>B. dermatitidis</em></td>
<td>2</td>
<td>0.1</td>
<td>0.5–1.0</td>
</tr>
<tr>
<td><em>P. brasiliensis</em></td>
<td>1</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td><em>S. schenckii</em></td>
<td>2</td>
<td>0.5–1.0</td>
<td>5.0–10.0</td>
</tr>
<tr>
<td><em>O. kairai</em></td>
<td>1</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>6</td>
<td>0.5</td>
<td>0.5–1.0</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>2</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td><em>C. pseudotropicalis</em></td>
<td>1</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td><em>C. guilliermondii</em></td>
<td>2</td>
<td>0.1–0.5</td>
<td>1.0</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>4</td>
<td>0.1–0.5</td>
<td>0.5–1.0</td>
</tr>
<tr>
<td><em>T. glabrata</em></td>
<td>1</td>
<td>0.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*MIC was measured after 2 to 3 days of incubation at 30°C and MFC after an additional 2 to 3 days of incubation following subculture to antibiotic-free media.*

**Table 2. In vitro susceptibility of yeast-like organisms to amphotericin B and AME by tube dilution technique**

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Strains tested</th>
<th>Susceptibility to Amphotericin B</th>
<th>Susceptibility to AME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MIC (µg/ml)*</td>
<td>MFC (µg/ml)</td>
</tr>
<tr>
<td><em>H. capsulatum</em></td>
<td>4</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td><em>B. dermatitidis</em></td>
<td>2</td>
<td>0.1</td>
<td>0.5–1.0</td>
</tr>
<tr>
<td><em>P. brasiliensis</em></td>
<td>1</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td><em>S. schenckii</em></td>
<td>2</td>
<td>0.5–1.0</td>
<td>10.0</td>
</tr>
<tr>
<td><em>O. kairai</em></td>
<td>1</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>6</td>
<td>0.5</td>
<td>0.5–1.0</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>2</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td><em>C. pseudotropicalis</em></td>
<td>1</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td><em>C. guilliermondii</em></td>
<td>2</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>4</td>
<td>0.1–0.5</td>
<td>0.5–1.0</td>
</tr>
<tr>
<td><em>T. glabrata</em></td>
<td>1</td>
<td>0.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*MIC was measured after 2 to 3 days of incubation at 30°C and MFC after an additional 2 to 3 days of incubation following subculture to antibiotic-free media.*

Amphotericin B and AME. Both drugs were inhibitory at a concentration of 0.5 µg/ml or less and fungicidal at 1.0 µg/ml. Among the dimorphic fungi, the filamentous forms of *S. schenckii* and *O. kairai* were less susceptible to both antibiotics. One isolate of *S. schenckii* was inhibited by 0.5 µg of both antibiotics per ml, whereas the other was inhibited by 5 µg of amphotericin B and 10 µg of AME per ml. The MFC of the two antibiotics for the mycelial form of *Sporothrix* was 10 µg/ml. For *O. kairai*, the MICs of amphotericin B and AME were 5 and 10 µg/ml, respectively. The fungicidal concentration of both antibiotics was 50 µg/ml.

The phycomycetes, *Aspergillus* and *Sepedonium*, were relatively more resistant to the antifungal activity of amphotericin B and AME, but amphotericin B was more effective against these fungi. For five isolates of *Rhizopus*, the MIC of amphotericin B was 0.5 µg/ml as compared with 5 µg/ml of AME. Amphotericin B inhibited both species of *Aspergillus* at 0.5 µg/ml, whereas 1 to 5 µg of AME per ml was required to inhibit growth. *Mucor pusillus* and *Syncephalastrum* sp., the other two phycomycetes, were more susceptible to amphotericin B than AME. The difference in the fungicidal activity of the two compounds was not as significant, and up to 50 µg of either antibiotic per ml was required for fungicidal action. The antibiotic susceptibilities of dermatophytes and dematacious fungi were comparable to those of phycomycetes. The MIC of amphotericin B ranged from 0.1 to 0.5 µg/ml.
and that of AME from 0.1 to 1.0 μg/ml. With the exception of *Phialophora compactum*, the MFCs of amphotericin B and AME ranged from 5 to 50 μg/ml. The MFC of amphotericin B for *P. compactum* was 0.5 μg/ml and that of AME was 1.0 μg/ml.

**DISCUSSION**

The results presented demonstrate that AME, a water-soluble derivative of amphotericin B, has significant antifungal activity against a variety of pathogenic and potentially pathogenic fungi. However, the antifungal activity of AME was slightly lower than that of the parent compound. Among the yeast-like organisms, only the yeast cells of *S. schenckii* were less susceptible than others to both antibiotics with MFCs of 5 to 10 μg/ml. The yeast cells of other fungi were killed at concentrations of 1 μg or less of either antibiotic per ml. The filamentous forms of *S. schenckii* and *O. kalraei* were more resistant than the filamentous forms of other dimorphic fungi to both drugs. The MFC of the two antibiotics for *S. schenckii* was 10 μg/ml and for *O. kalraei*, 50 μg/ml. The dermatophytes and dematacious and other potentially pathogenic fungi were inhibited fairly well by both drugs, but up to 50 μg/ml were required for fungicidal action. These findings extend the earlier report of the antifungal activity of AME against *S. cerevisiae* and *C. albicans* (1).

*S. schenckii* and *Allescheria boydii* have been shown to be more resistant than other fungi to amphotericin B (8). The MFC of amphotericin B for *S. schenckii* was 25 μg/ml, and it was greater than 100 μg/ml for *A. boydii*. In the present study, amphotericin B and its water-soluble derivative (AME) also were less effective against *S. schenckii*, *O. kalraei*, phycomycetes, and dematacious and certain other opportunistic fungi. The reasons for the differences in the susceptibility of different fungi for amphotericin B and AME are not known.

The lower antifungal activity of AME as compared with the parent compound could be due to lower stability or modification of chemical structure necessary for antifungal activity. Our observations on the inhibitory action of amphotericin B and AME on macromolecular synthesis by yeast cells of *H. capsulatum* support the contention that the lower antifungal activity of AME is due to decreased stability (unpublished data). Both antibiotics inhibited the incorporation of radioactive precursors into

**Table 3. In vitro susceptibility of fungi in mycelial form to amphotericin B and AME by agar dilution technique**

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>MIC (μg/ml)</th>
<th>MFC (μg/ml)</th>
<th>MIC (μg/ml)</th>
<th>MFC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. capsulatum</em></td>
<td>0.1-0.5</td>
<td>0.5-5.0</td>
<td>0.1-1.0</td>
<td>0.5-5.0</td>
</tr>
<tr>
<td><em>H. dubosii</em></td>
<td>0.1</td>
<td>0.5</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td><em>B. dermatitidis</em></td>
<td>0.1-0.5</td>
<td>0.5-1.0</td>
<td>0.1-0.5</td>
<td>0.5-1.0</td>
</tr>
<tr>
<td><em>S. schenckii</em></td>
<td>0.5-5.0</td>
<td>10.0</td>
<td>0.5-10.0</td>
<td>10.0</td>
</tr>
<tr>
<td><em>O. kalraei</em></td>
<td>5.0</td>
<td>50.0</td>
<td>10.0</td>
<td>50.0</td>
</tr>
<tr>
<td><em>P. brasiliensis</em></td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td><em>C. immitis</em></td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td><em>Rhizopus sp.</em></td>
<td>0.5</td>
<td>50.0</td>
<td>5.0</td>
<td>50.0</td>
</tr>
<tr>
<td><em>R. arrhizus</em></td>
<td>0.5</td>
<td>50.0</td>
<td>5.0</td>
<td>50.0</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>0.5</td>
<td>10.0</td>
<td>5.0</td>
<td>10.0</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>0.5</td>
<td>5.0</td>
<td>1.0</td>
<td>5.0</td>
</tr>
<tr>
<td><em>M. pusillus</em></td>
<td>0.1</td>
<td>10.0</td>
<td>0.5</td>
<td>50.0</td>
</tr>
<tr>
<td><em>Sepedonium sp.</em></td>
<td>0.5</td>
<td>1.0</td>
<td>0.5</td>
<td>10.0</td>
</tr>
<tr>
<td><em>Synechocystis</em></td>
<td>0.1</td>
<td>10.0</td>
<td>0.5</td>
<td>50.0</td>
</tr>
<tr>
<td><em>P. compactum</em></td>
<td>0.1</td>
<td>0.5</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td><em>P. dermatitidis</em></td>
<td>0.5</td>
<td>50.0</td>
<td>0.5</td>
<td>50.0</td>
</tr>
<tr>
<td><em>P. verrucosa</em></td>
<td>0.5</td>
<td>5.0</td>
<td>1.0</td>
<td>50.0</td>
</tr>
<tr>
<td><em>Cladosporium sp.</em></td>
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<td>50.0</td>
<td>1.0</td>
<td>50.0</td>
</tr>
<tr>
<td><em>M. canis</em></td>
<td>0.5</td>
<td>5.0</td>
<td>1.0</td>
<td>10.0</td>
</tr>
<tr>
<td><em>M. gypseum</em></td>
<td>0.5</td>
<td>5.0</td>
<td>1.0</td>
<td>5.0</td>
</tr>
<tr>
<td><em>T. mentagrophytes</em></td>
<td>0.5</td>
<td>5.0</td>
<td>0.5</td>
<td>10.0</td>
</tr>
<tr>
<td><em>T. rubrum</em></td>
<td>0.5</td>
<td>10.0</td>
<td>0.5</td>
<td>10.0</td>
</tr>
<tr>
<td><em>T. simii</em></td>
<td>0.5</td>
<td>10.0</td>
<td>0.5</td>
<td>10.0</td>
</tr>
</tbody>
</table>

* MIC was measured after 2 to 7 days of incubation at 30 C and MFC after an additional 2 to 7 days of incubation following subculture to antibiotic-free media.
Table 4. In vitro susceptibility of fungi in mycelial form to amphotericin B and AME by tube dilution techniques

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Strains tested</th>
<th>Susceptibility to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Amphotericin B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MIC (µg/ml)*</td>
</tr>
<tr>
<td>H. capsulatum</td>
<td>6</td>
<td>0.1-0.5</td>
</tr>
<tr>
<td>H. duboisii</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>B. dermatitidis</td>
<td>2</td>
<td>0.1-0.5</td>
</tr>
<tr>
<td>S. schenkii</td>
<td>2</td>
<td>0.5-1.0</td>
</tr>
<tr>
<td>O. kaira</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>P. brasiliensis</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>C. immitis</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>Rhizopus sp.</td>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td>R. arrhizus</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>A. niger</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>A. fumigatus</td>
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<td>0.5</td>
</tr>
<tr>
<td>M. pusillus</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Sepedonium sp.</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Syncephalastrum sp.</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>P. compactum</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>P. dermatitidis</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>P. verrucosum</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Cladosporum sp.</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>M. canis</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>M. gypseum</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>T. mentagrophytes</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>T. rubrum</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>T. simii</td>
<td>1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

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We are indebted to William Brown, the Squibb Institute for Medical Research, for the supply of amphotericin B and to Carl Schaffner, Rutgers University, for amphotericin B methyl ester.

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ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grants AI-11084 from the National Institute of Allergy and Infectious Diseases and FR-7058 from the Division of Research Facilities and Resources.

ribonucleic acid and protein at 0.05 µg/ml, and the degree of inhibition attained by the two antibiotics was comparable.

Sterols and serum have been shown to interfere with the antifungal activity of polyene antibiotics (2, 4). Therefore, synthetic media free of these inhibitors, ones that adequately supported the growth of the test organisms, were used in the present study. The agar dilution technique appears preferable because it permits reliable testing of higher concentrations of antibiotics as compared with the broth dilution technique.

The retention of the wide spectrum of antifungal activity of amphotericin B and the water solubility and the significant lower toxicity of AME in experimental animals (3) warrant evaluation of its chemotherapeutic activity against a variety of experimental fungal infections.

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