Investigation of the Efficacy of Micafungin in the Treatment of Histoplasmosis

Using Two North American Strains of *Histoplasma Capsulatum*

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**Running title:** Micafungin Histoplasmosis

Word count: abstract: 43, body text: 909

Tables: 2  Figures 2

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Abstract.

Micafungin alone and combined with liposomal amphotericin B was evaluated against two strains of *Histoplasma capsulatum*. Micafungin was active *in vitro* against the mould but not the yeast form but was ineffective *in vivo*. Micafungin appears to be ineffective in treatment of histoplasmosis.
Histoplasmosis a life-threatening infection in patients with weakened immunity. While lipid formulations of amphotericin B and itraconazole are the preferred treatments, occasionally other agents are needed. *In vitro* and animal studies showed conflicting results using echinocandins in the treatment of histoplasmosis (4, 8, 11, 17).

Caspofungin demonstrated limited efficacy in a pulmonary (11) but was effective in an intravenous murine model of histoplasmosis (8). Reasons for the conflicting findings are unknown, but may include the use of different *Histoplasma* isolates, routes of infections, or mouse strains (12). The objective of this study was to examine the efficacy of micafungin (MFG) as compared to liposomal amphotericin B (L-AMB) against the two strains of *H. capsulatum* used in the conflicting studies.

The *H. capsulatum* isolates were IU-CT (3, 11) and 94-255 (8). Genotyping using *YPS3* (1, 9) showed the 94-255 isolate did not express YPS3, and thus was a class 1 strain whereas the IU-CT isolate expressed YPS3, which is characteristic for class 2 (Figure 1-A), the predominant type in North America (20).

The minimal inhibitory concentrations (MIC) or minimal effective concentrations (MEC) of the two strains were compared. The MIC for the yeast form was determined as the dilution at which there was no visible growth (6, 11, 16, 19) and the MEC for the mould was the lowest concentration of drug causing growth of small, rounded, compact hyphal forms, as compared to control wells (5). The Fractional Inhibitory Concentration Index (FICI) was determined using the checkerboard method to classify drug interaction between MFG and L-AMB: synergy (FICI ≤ 0.5), antagonism (FICI > 4.0) and no interaction (FICI > 0.5–4.0) (14) .
L-AMB was highly effective \textit{in vitro} against the yeast and mould forms of both strains whereas MFG was effective only against the mould forms. L-AMB and MFG displayed no interaction with either mould or yeast forms (Table 1), consistent with earlier reports (11, 13).

Six-week old C57Bl6 mice (Jackson Laboratories, Bar Harbor, ME) were infected intranasally with $1 \times 10^7$ yeasts, based on preliminary experiments using the IU-CT class-2 isolate ($1-2 \times 10^7$ caused 80-100% mortality). All experiments were performed twice to confirm reproducibility, and treatment groups contained 9 to 10 mice. Treatment was started on day four of infection and continued for ten days. Groups were as follows:

- group 1, L-AMB 0.5 mg/kg intraperitoneally (i.p.) every other day (q.o.d.);
- group 2, L-AMB 2.0 mg/kg i.p. q.o.d.;
- group 3, MFG 10 mg/kg i.p. once daily (q.d.);
- group 4, L-AMB 0.5 mg/kg i.p. q.o.d. and MFG 10 mg/kg i.p. q.d.;
- group 5, L-AMB 2.0 mg/kg i.p. q.o.d. and MFG 10 mg/kg i.p. q.d.;
- group 6, vehicle control (5% dextrose-water solution) i.p. q.d.

Twenty four hours after the last antifungal injection, mice were sacrificed, and lungs and spleens were removed aseptically and weighed. Quantitative culture of organ homogenates to measure fungal burden was expressed as CFU per gram of tissue.

All mice infected with the 94-255 class 1 isolate survived to day 15 including those treated with control vehicle. In preliminary experiments, mice survived infection with up to $1 \times 10^8$ class-1 yeasts, prohibiting analysis of mortality. Compared to vehicle treated mice, lung fungal burden was lower only in mice treated with L-AMB 2.0 mg/kg alone ($p=0.013$). Mice treated with 2 mg/kg L-AMB had lower lung fungal burden than those
receiving the combination of L-AMB 2.0 mg/kg and MFG 10 mg/kg (p=0.045), suggesting in vivo antagonism (Figure 2-A). Spleen fungal burden was lower in all groups (p<0.05) except for MFG alone (p=0.54).

Following infection with the IU-CT class 2 isolate, only two mice (20%) receiving MFG and four (40%) vehicle control mice survived, compared to all ten receiving L-AMB 2 mg/kg, nine (90%) receiving L-AMB, nine (90%) receiving L-AMB 2.0 mg/kg with MFG, nine (90%) receiving L-AMB 0.5 mg/kg alone and seven (70%) receiving L-AMB 0.5 mg/kg with MFG (Figure 1-B). Cox proportional-hazards regression analysis showed treatment with MFG associated with reduced survival (p<0.0001). L-AMB 2.0 mg/kg alone or combined with MFG appeared to reduce fungal burden in the lung and spleen, but too few vehicle control mice survived for statistical analysis (Figure 2-B). YPS3 was expressed in the IU-CT class-2 strain, perhaps contributing to the higher mortality, as YPS3 is a virulence factor associated with extrapulmonary dissemination (2, 10).

Since echinocandins exert their antifungal activity by inhibiting 1,3 beta-D glucan synthase (7), in vitro activity against the mould but not the yeast might be related to the difference in the glucan composition between the two growth forms. The cell walls of the mould contain mainly beta-1,3 glucan but the yeast form contain predominantly alpha-glucan (13, 15, 18). (1,3)-beta-D-Glucan (BG) assay was performed at the Associates of Cape Cod, Inc., (East Falmouth) on pooled mouse serum samples obtained on day 15 after infection, and on culture supernatant from the yeast of both isolates using the same methods as for the in-vitro susceptibility testing. BG was not detected in pooled serum from mice infected with either strain and treated with either L-AMB or MFG, or
uninfected control mice. Similarly, in vitro, BG was undetected in the yeast culture supernatant that contained either L-AMB or MFG but was detected in control wells (table-2).

In conclusion, while L-AMB was highly effective in vitro in both yeast and mould forms; MFG was effective only against the mould and was not effective in vivo with either strain of H. capsulatum. No in vitro interaction was detected between L-AMB and MFG. The echinocandins appear ineffective in the treatment of histoplasmosis.
Acknowledgements:

This work was partly supported by a VA Career Development Award (CDA-2) to C.A.H and an unrestricted grant by Astellas Pharma Global Development, Inc to L.J.W. Part of this work was supported by the University of Texas-Pan American Faculty Research Council Grant Award (FRC – Molecular Strategy 135BIOL07 to R.Z.).
References:


Table 1. In vitro susceptibility of yeast and mold forms of both strains of *H. capsulatum* to liposomal amphotericin B and micafungin.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Measurement</th>
<th>Mould</th>
<th>Yeast</th>
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<tr>
<td></td>
<td></td>
<td>Class 1</td>
<td>Class 2</td>
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<tr>
<td>L-AMB</td>
<td>MIC</td>
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<tr>
<td>MFG</td>
<td>MEC or MIC</td>
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<tr>
<td>L-AMB + MFG</td>
<td>FIC index</td>
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Table 2: Beta glucan concentration in infected mouse serum and *in vitro* yeast culture supernatants

<table>
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<th>Class-2</th>
<th>Uninfected</th>
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<tr>
<td><strong>Infected mouse serum, day 15 of infection</strong></td>
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<td>&lt;31</td>
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<tr>
<td>L-AMB 2.0 mg/kg qod</td>
<td>&lt; 31</td>
<td>&lt; 31</td>
<td></td>
</tr>
<tr>
<td>MFG10 mg/kg q.d</td>
<td>&lt; 31</td>
<td>&lt; 31</td>
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<tr>
<td>No drug controls</td>
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<td>Interference, no result</td>
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<tr>
<td></td>
<td>Class-1</td>
<td>Class-2</td>
<td>Uninoculated</td>
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<tr>
<td><strong>In vitro culture supernatant (yeast form)</strong></td>
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<td>&lt;31</td>
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<td>L-AMB 2.0 mg/kg qod</td>
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<tr>
<td>MFG10 mg/kg q.d</td>
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<td>No drug controls</td>
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Figure 1- A: Genotyping of *H. capsulatum* isolates based on HaeIII-generated RFLP of the *YPS3* gene. L indicates 100 base pair ladder; lane 1 represents class 1 isolate (Downs); lane 2 is a class 1 isolate (UCLA531S); lane 3 is a class 2 isolate (G217B); lane 4 is a class 3 isolate (G186AS); lane 5 is the 94-255 isolate and lane 6 is the IU-CT isolate. B: Survival of mice infected with *H. capsulatum* class 2 at $1 \times 10^7$ intranasally.
Figure 2. Quantitative culture results for lungs (full circles) and spleens (open circles) from mice that were sacrificed 14 days after infection intranasally with $10^7$ yeasts IU-CT (A) and 94-255 (B). Each data point represents one animal. Horizontal bars represent the median of the corresponding data column. Lower limit of detection (LOD) is 20 CFU/g of organ tissue. P values using paired comparisons (Mann-Whitney test), after adjustment by stepdown Bonferroni multiple comparison procedure, are listed above the columns: 

# $p=0.013$ compared to vehicle treated mice, ** $p=0.045$ compared to mice treated with L-L-AMB 2.0 mg/kg, * $p<0.005$ compared to vehicle treated mice.