

# Investigation of the Efficacy of Micafungin in the Treatment of Histoplasmosis Using Two North American Strains of *Histoplasma capsulatum*<sup>∇</sup>

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**Micafungin alone and combined with liposomal amphotericin B was evaluated against two strains of *Histoplasma capsulatum*. Micafungin was active *in vitro* against the mold but not the yeast form but was ineffective *in vivo*. Micafungin appears to be ineffective in treatment of histoplasmosis.**

Histoplasmosis is 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 model (11) but was effective in an intravenous murine model of histoplasmosis (8). The reasons for the conflicting findings are unknown but may include the use of different *Histoplasma* isolates, routes of infection, or mouse strains (12). The objective of this study was to examine the efficacy of micafungin (MFG) compared to that of lipo-

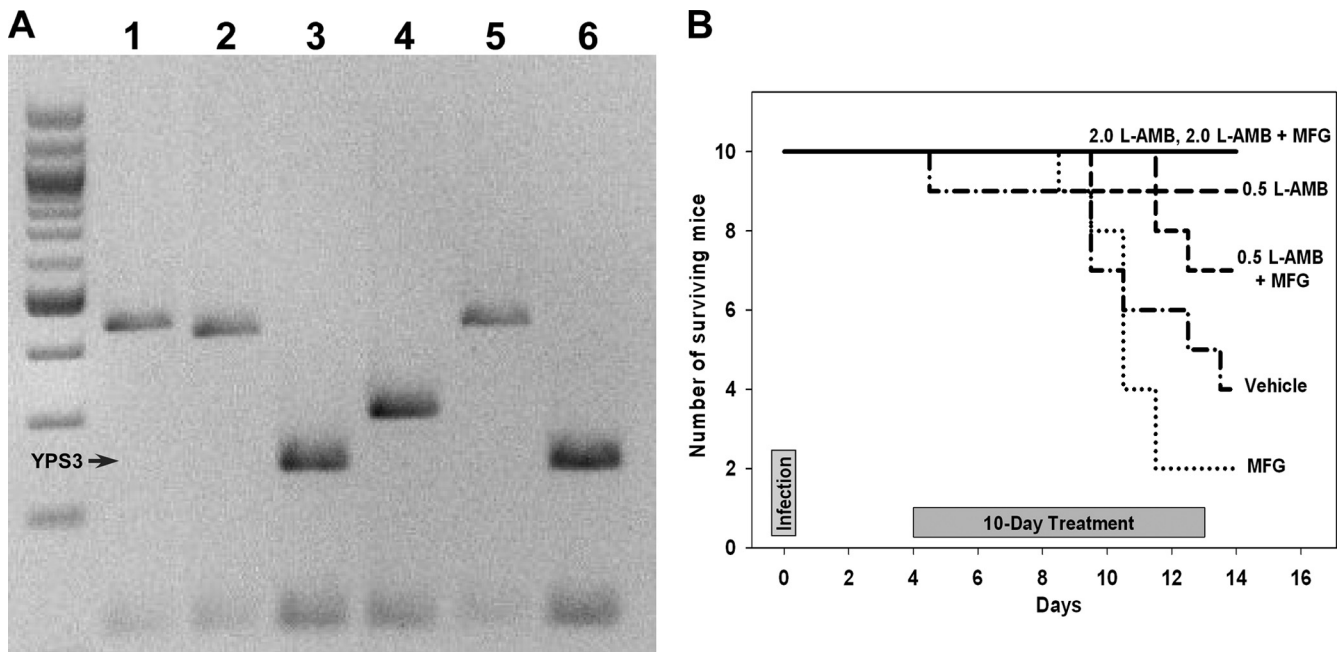


FIG. 1. (A) Genotyping of *H. capsulatum* isolates based on HaeIII-generated restriction fragment length polymorphism (RFLP) of the *YPS3* gene. The first lane is a 100-bp ladder; lane 1, class 1 isolate (Downs); lane 2, class 1 isolate (UCLA531S); lane 3, class 2 isolate (G217B); lane 4, class 3 isolate (G186AS); lane 5, 94-255 isolate; lane 6, IU-CT isolate. (B) Survival of mice infected with *H. capsulatum* class 2 at  $1 \times 10^7$  yeasts intranasally. 2.0, 0.5, dose in mg/kg.

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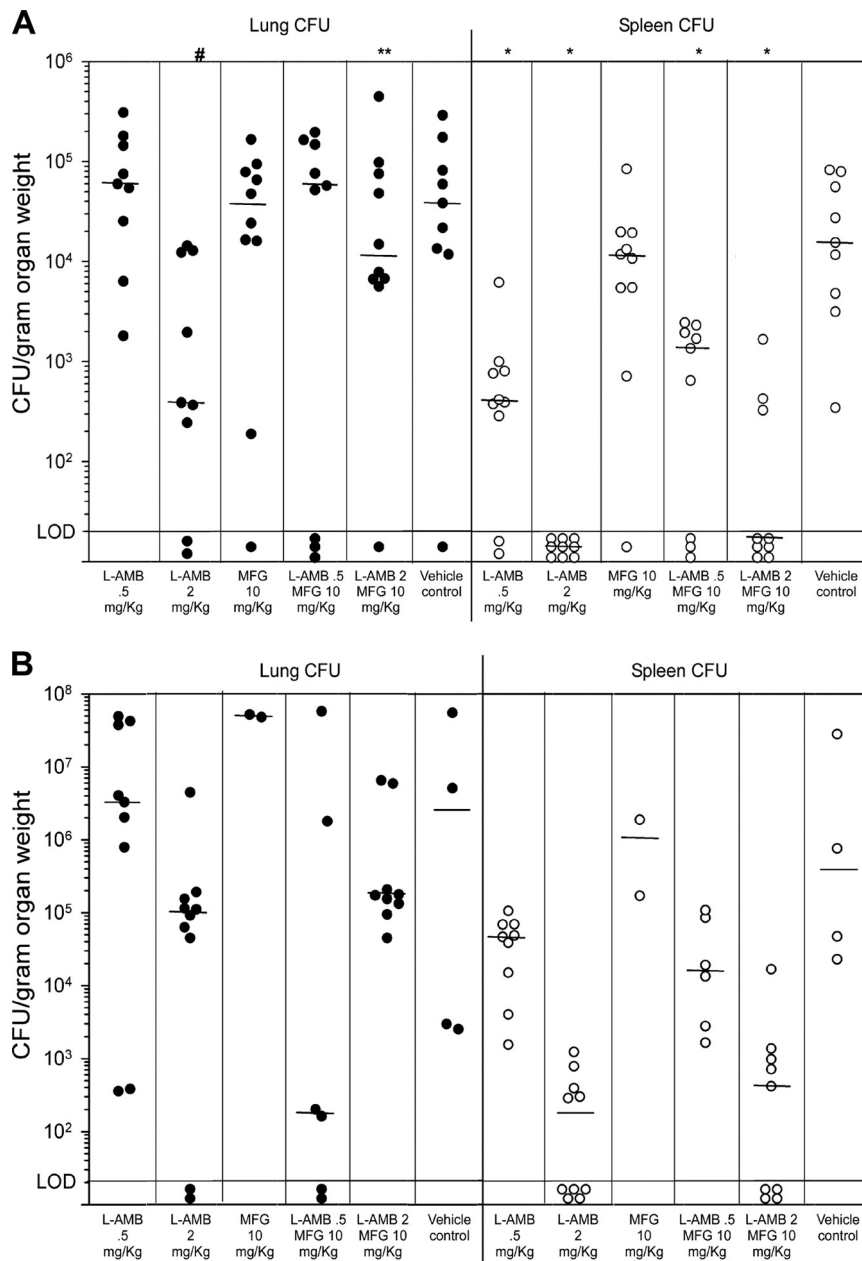


FIG. 2. Quantitative culture results for lungs (filled circles) and spleens (open circles) from mice that were sacrificed 14 days after intranasal infection with  $10^7$  IU-CT (A) and 94-255 (B) yeasts. Each data point represents the result for one animal. Horizontal bars represent the medians of the corresponding data columns. Lower limit of detection (LOD) is 20 CFU/g of organ tissue. *P* values using paired comparisons (Mann-Whitney test), after adjustment by step-down Bonferroni multiple-comparison procedure, are listed above the columns as follows: #, *P* = 0.013 compared to vehicle-treated mice; \*\*, *P* = 0.045 compared to mice treated with L-AMB at 2.0 mg/kg; \*, *P* < 0.005 compared to vehicle-treated mice.

somal amphotericin B (L-AMB) against the two strains of *Histoplasma 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 that 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 (Fig. 1A), the predominant type in North America (20).

The MICs or minimal effective concentrations (MECs) 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 mold was the lowest concentration of drug causing growth of small, rounded, compact hyphal forms, compared to the results in control wells (5). The fractional inhibitory concentration index (FICI) was determined using the checkerboard method to classify drug interaction between MFG and L-AMB, as follows: synergy (FICI  $\leq$  0.5), antagonism (FICI > 4.0), and no interaction (FICI > 0.5 to 4.0) (14).

L-AMB was highly effective *in vitro* against the yeast and

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

<i>H. capsulatum</i> form and class	MIC of L-AMB (µg/ml)	MEC or MIC of MFG (µg/ml)	FIC index for L-AMB + MFG
Mold			
1	0.25	0.06	0.75
2	0.25	0.06	0.75
Yeast			
1	0.25	>64	0.62
2	0.125	>64	0.62

mold forms of both strains, whereas MFG was effective only against the mold forms. L-AMB and MFG displayed no interaction with either the mold or yeast forms (Table 1), consistent with earlier reports (11, 13).

Six-week-old C57BL/6 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 ( $1 \times 10^7$  to  $2 \times 10^7$  yeasts caused 80 to 100% mortality). All experiments were performed twice to confirm reproducibility, and treatment groups contained 9 or 10 mice. Treatment was started on day 4 of infection and continued for 10 days. The groups were as follows: group 1, L-AMB at 0.5 mg/kg of body weight intraperitoneally (i.p.) every other day (q.o.d.); group 2, L-AMB at 2.0 mg/kg i.p. q.o.d.; group 3, MFG at 10 mg/kg i.p. once daily (q.d.); group 4, L-AMB at 0.5 mg/kg i.p. q.o.d. and MFG at 10 mg/kg i.p. q.d.; group 5, L-AMB at 2.0 mg/kg i.p. q.o.d. and MFG at 10 mg/kg i.p. q.d.; and 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 the vehicle control. In preliminary experiments, mice survived infection with up to  $1 \times 10^8$  class 1 yeasts, prohibiting analysis of mortality. Compared to that in vehicle-treated mice, the lung fungal burden was lower only in mice treated with L-AMB at 2.0 mg/kg alone ( $P = 0.013$ ). Mice treated with 2 mg/kg L-AMB had lower lung fungal burdens than those receiving the combination of L-AMB at 2.0 mg/kg and MFG at 10 mg/kg ( $P = 0.045$ ), suggesting *in vivo* antagonism (Fig. 2A). The 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 2 mice (20%) receiving MFG and 4 (40%) vehicle control mice survived, compared to all 10 receiving L-AMB at 2 mg/kg, 9 (90%) receiving L-AMB, 9 (90%) receiving L-AMB at 2.0 mg/kg with MFG, 9 (90%) receiving L-AMB at 0.5 mg/kg alone, and 7 (70%) receiving L-AMB at 0.5 mg/kg with MFG (Fig. 1B). Cox proportional hazards regression analysis showed treatment with MFG to be associated with reduced survival ( $P < 0.0001$ ). L-AMB at 2.0 mg/kg alone or combined with MFG appeared to reduce the fungal burden in the lung and

TABLE 2. Beta glucan concentrations in infected mouse serum and *in vitro* yeast culture supernatants

Specimen and treatment	Concn of beta glucan (pg/ml)		
	Class 1 <i>H. capsulatum</i>	Class 2 <i>H. capsulatum</i>	Uninfected or uninoculated control
Infected mouse serum, day 15 of infection			<31
L-AMB at 2.0 mg/kg q.o.d.	<31	<31	
MFG at 10 mg/kg q.d.	<31	<31	
No-drug controls	125	Interference, no result	
<i>In vitro</i> culture supernatant (yeast form)			<31
L-AMB at 2.0 mg/kg q.o.d.	<31	<31	
MFG at 10 mg/kg q.d.	<31	<31	
No-drug controls	104	>500	

spleen, but too few vehicle control mice survived for statistical analysis (Fig. 2B). 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 (BG) synthase (7), *in vitro* activity against the mold but not the yeast might be related to the difference in the glucan composition between the two growth forms. The cell walls of the mold contain mainly beta-1,3 glucan, but the yeast form contains predominantly alpha-glucan (13, 15, 18). The BG assay was performed by Associates of Cape Cod, Inc. (East Falmouth, MA), 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 from uninfected control mice. Similarly, *in vitro*, BG was not detected 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 mold forms, MFG was effective only against the mold *in vitro* 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 to be ineffective in the treatment of histoplasmosis.

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