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In Vitro and *In Vivo* Antifungal Activity of AmBisome Compared to Conventional Amphotericin B and Fluconazole against *Candida auris*

Janet Herrada,[a,b] Ahmed Gamal,[a,b] Lisa Long,[a,b] Sonia P. Sanchez,[c] Thomas S. McCormick,[a,b] Mahmoud A. Ghannoum[a,b]

[a]Center for Medical Mycology, University Hospitals Cleveland Medical Center, Cleveland, Ohio, USA
[b]Case Western Reserve University, Cleveland, Ohio, USA
[c]Gilead Sciences Europe Ltd., Uxbridge, United Kingdom

ABSTRACT Antifungal activity of AmBisome against *Candida auris* was determined *in vitro* and *in vivo*. AmBisome showed MIC_{50} and MIC_{90} values of 1 and 2 μg/ml, respectively. Unlike conventional amphotericin B, significant *in vivo* efficacy was observed in the AmBisome 7.5 mg/kg treatment group in survival and reduction of kidney tissue fungal burden compared to the untreated group. Our data show that AmBisome has significant antifungal activity against *C. auris* infection *in vitro* and *in vivo*.

KEYWORDS *Candida auris*, multidrug resistant, AmBisome, conventional amphotericin B, fluconazole, susceptibility testing, mouse models

Candida auris is an emerging multidrug-resistant yeast that has been associated with nosocomial infection worldwide (1), with a high infection rate in elderly patients and those with underlying illnesses (2–7). Critically, most *C. auris* isolates are resistant to at least two of the three main antifungal classes (azoles, polyenes, echinocandins), with some strains of *C. auris* exhibiting pan resistance (8–10). Although resistance of *C. auris* strains to conventional amphotericin B (CAmphoB) has been reported, activity of the liposomal preparation (AmBisome) against this yeast was not reported. Here, the *in vitro* and *in vivo* activity of AmBisome compared with CAmphoB and fluconazole against *C. auris* was investigated.

Clinical *C. auris* isolates ($n = 35$) were used in this study. Susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) document M27-A4 (11). AmBisome was highly active against most of the tested strains (29/35, 83%), with an MIC range between 0.25 and 2 μg/ml and MIC_{50} and MIC_{90} values of 1 and 2 μg/ml (Table 1). In contrast, CAmphoB showed an MIC range of 1 to 4 μg/ml and MIC_{50} and MIC_{90} values of 4 μg/ml for both. Fluconazole was the least active, with most *C. auris* isolates demonstrating high resistance to this antifungal (12). Fluconazole showed MIC ranges of 0.25 to >64 μg/ml, with MIC_{50} and MIC_{90} values of 64 μg/ml.

Using tentative breakpoints suggested by the CDC, we showed that 77% of the isolates tested were resistant to CAmphoB, with MIC values of ≥2 μg/ml, whereas only 17% of the examined isolates showed resistance to AmBisome.

In vivo testing was performed using a previously described disseminated *C. auris* infection model (13). All procedures were performed in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and approval of the Case Western Reserve University Institutional Animal Care and Use Committee (IACUC). Female BALB/c mice (weighing ~20 g; Charles River Laboratories, Wilmington, MA) were used in the study.

C. auris (MRL 35368) with *in vitro* MICs for AmBisome, CAmphoB, and fluconazole of 1, 4, and >64 μg/ml, respectively, was used to challenge the mice. Treatment was initiated 2 h

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Address correspondence to Mahmoud A. Ghannoum, mag3@case.edu.

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TABLE 1 MICs for AmBisome, amphotericin B, and fluconazole against *C. auris*

Parameter for *C. auris* ($n = 35$)	MIC (μg/ml) for:		
	AmBisome	Amphotericin B	Fluconazole
Range	0.25–2	1–4	0.25 to >64
MIC_{50}	1	4	64
MIC_{90}	2	4	>64

postchallenge. Treatment groups consisted of once-daily dosing of AmBisome at 3.5, 5, and 7.5 mg/kg; CAmphoB at 0.75 mg/kg administered intraperitoneally; and fluconazole at 25 mg/kg administered orally. Untreated control animals were included. Efficacy endpoints were animal survival and kidney fungal load.

Survival was monitored for 14 days postinoculation. The AmBisome 7.5 mg/kg treatment group showed 90% survival at 14 days postinoculation (Fig. 1), whereas CAmphoB- and fluconazole-treated groups showed 20% and 50% survival, respectively. The AmBisome 7.5 mg/kg group demonstrated significant survival compared with the untreated control group ($P < 0.001$) and had significantly higher survival rates than either the CAmphoB or fluconazole group ($P = 0.003$ and 0.025, respectively). The AmBisome formulation allows the drug to remain within the liposomes until it adheres to the fungal cell wall, where it enters the fungus intact, preventing the drug from interacting with mammalian cells to exert its toxic effects (14, 15).

Five mice from each group were euthanized 1 day after treatment (day 8) to determine kidney fungal burden. Kidneys were removed aseptically and weighed, homogenized in 1 ml of phosphate-buffered saline, serially diluted, plated onto potato dextrose agar (Becton, Dickinson and Company, Sparks, MD), and cultured at 37°C for 48 h to determine CFU per gram. The tissue fungal burden was expressed as CFU per gram of tissue. Efficacy of AmBisome was evaluated as a reduction in $\log_{10}$ CFU compared with other tested groups.

Table 2 shows the kidney tissue fungal burden of mice challenged with *C. auris*. As expected, the mice in the untreated control group showed the highest kidney tissue fungal burden (8.64 ± 0.7 log CFU/g ± SD). The AmBisome 7.5 mg/kg group demonstrated

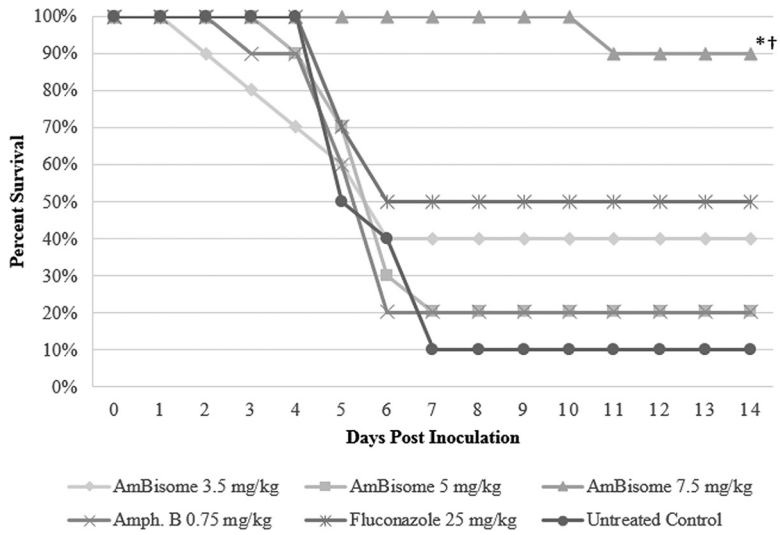

FIG 1 Survival curves in BALB/c mice infected with *C. auris* (3×10^7). Treatments were administered by intraperitoneal injection for AmBisome and conventional amphotericin B and orally for fluconazole ($n = 10$/group). Treatment consisted of once-daily dosing starting 2 h postchallenge and continuing for 7 days. Survival was monitored until day 14. Survival was plotted by Kaplan-Meier analysis, and differences in the percent survival among groups were analyzed by log-rank test and Fisher exact test. *, $P \leq 0.001$ versus untreated control; †, $P < 0.05$ versus conventional amphotericin B and fluconazole, respectively.

TABLE 2 Average kidney tissue fungal burden compared with untreated control

Treatment group	Kidney tissue fungal burden (avg log CFU/g ± SD)	*P* value
Untreated control	8.64 ± 0.7	
AmBisome (mg/kg)		
3.5	6.54 ± 1.2	0.387
5	5.88 ± 2.2	0.069
7.5	5.55 ± 0.6	0.028
Amphotericin B 0.75 mg/kg	6.18 ± 1.6	0.356
Fluconazole 25 mg/kg	5.46 ± 1.4	0.022

a tissue fungal burden of 5.55 ± 0.6 log CFU/g. The AmBisome 5 and 3.5 mg/kg treatment groups showed tissue fungal burdens of 5.88 ± 2.2 and 6.54 ± 1.2 log CFU/g, respectively. These data show that AmBisome reduced tissue burden in a dose-dependent manner, with AmBisome 7.5 mg/kg reaching a significant reduction in tissue fungal burden ($P = 0.028$). The 5- and 3.5-mg/kg AmBisome arms showed a trend toward reducing kidney tissue fungal burden compared with the untreated control group, which did not reach statistical significance ($P = 0.069$ and 0.387, respectively). In contrast to AmBisome 7.5 mg/kg, CAmphoB 0.75 mg/kg did not demonstrate significant efficacy against *C. auris* (6.18 ± 1.6 CFU/g; $P = 0.356$). However, fluconazole 25 mg/kg treatment showed significant reduction in fungal tissue burden (5.46 ± 1.4 CFU/g) compared with the untreated control ($P = 0.022$). No statistical difference in fungal tissue burden was noted between the AmBisome 7.5 mg/kg and fluconazole treatment groups ($P > 0.05$).

AmBisome 7.5 mg/kg demonstrated a significant reduction in CFU in the kidneys, with a better survival rate than CAmphoB and fluconazole. This could be explained by the ability of AmBisome to distribute to infected tissues at levels above the MIC values. AmBisome also demonstrated slow tissue clearance rates (16).

Interestingly, the isolate used in our animal model demonstrated resistance to fluconazole when tested *in vitro*, whereas fluconazole *in vivo* showed a significant reduction in CFU in the kidneys. This difference could be because susceptibility tests are designed to test the activity of antifungals in a static test situation. These conditions do not mimic the *in vivo* situation in that many factors may influence the intrinsic antifungal properties, including the underlying condition and immunological status of the animal model (17). Although fluconazole demonstrated activity in reducing the kidney tissue burden, it was not as effective as AmBisome in enhancing animal survival rates ($P = 0.025$ versus AmBisome 7.5 mg/kg).

The underlying mechanisms for AmBisome's higher *in vitro* and *in vivo* activity against *C. auris* compared with CAmphoB are unknown. Recently, Walker et al. (14) reported that the viscoelastic properties of the *Candida albicans* and *Cryptococcus neoformans* cell wall allowed for travel of AmBisome as intact liposome vesicles. At the target site, the higher affinity of amphotericin B for fungal ergosterol over the lipid carrier and the availability of extracellular yeast and host lipases facilitated the release of amphotericin B from the lipid complex, where it binds to the cell membrane of the fungal pathogen (16). Additional evidence differentiating liposomal amphotericin B formulations from CAmphoB can be gleaned from the findings that lipid formulations are more efficient at inhibiting *Candida* biofilms than CAmphoB *in vitro* (18, 19) and in a rabbit model of catheter-associated *C. albicans* biofilm infection (20, 21).

Taken together our findings show that AmBisome possesses significant antifungal activity against *C. auris in vitro* and *in vivo* compared with CAmphoB and fluconazole.

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