Assessing the Antifungal Activity, Pharmacokinetics, and Tissue Distribution of Amphotericin B Following the Administration of Abelcet® and AmBisome® in Combination with Caspofungin to Rats Infected with *Aspergillus fumigatus*

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ABSTRACT: The purpose of this study was to assess the antifungal activity, pharmacokinetics, and tissue distribution of amphotericin B (AmpB) following the administration of Abelcet® and AmBisome® alone and in combination with Caspofungin® to rats infected with *Aspergillus fumigatus*. *Aspergillus fumigatus* inoculum ($2.1-2.5 \times 10^7$ colony forming units [CFU]) was injected via the jugular vein; 48 h later male albino Sprague–Dawley rats (350–400 g) were administered either a single intravenous (i.v.) dose of Abelcet® (5 mg AmpB/kg; $n = 6$), AmBisome® (5 mg AmpB/kg; $n = 6$), Caspofungin® (3 mg/kg; $n = 5$), Abelcet® (5 mg AmpB/kg) plus Caspofungin® (3 mg/kg) ($n = 6$), AmBisome® (5 mg AmpB/kg) plus Caspofungin® (3 mg/kg) ($n = 7$), or physiologic saline (non-treated controls; $n = 6$) once daily for 4 days. Antifungal activity was assessed by organ CFU concentrations and plasma galactomannan levels. Plasma and tissue samples were taken from each animal for AmpB pharmacokinetic analysis and tissue distribution determinations. Abelcet® treatment significantly decreased total fungal CFU concentrations recovered in all the organs added together by 73% compared to non-treated controls. Ambisome® treatment significantly decreased total fungal CFU concentrations recovered in all the organs added together by 69% compared to non-treated controls. Caspofungin® treatment significantly decreased total fungal CFU concentrations recovered in all the organs added together by 80% compared to non-treated controls. Abelcet® plus Caspofungin® treatment significantly decreased total fungal CFU concentrations recovered in all the organs added together by 81% compared to non-treated controls. Ambisome® plus Caspofungin® treatment significantly decreased total fungal CFU concentrations recovered in all the organs added together by 98% compared to non-treated controls. Abelcet® treatment significantly decreased plasma galactomannan levels by 50 and 75% 96 h following the initiation of treatment in the absence and presence of Caspofungin co-therapy, respectively. AmBisome® treatment significantly decreased plasma galactomannan levels by 73 and 78% 96 h following the initiation of treatment in the absence and presence of Caspofungin co-therapy, respectively. Co-administration of Caspofungin with Abelcet and AmBisome

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did not significantly alter the plasma concentration-time profile, pharmacokinetic parameters, and tissue distribution of AmpB. Taken together, our findings suggest that an alternative mechanism, possibly at the cellular level rather than altered AmpB disposition, may be an explanation for the differences in organ CFU concentrations following Abelcet® plus Caspofungin versus AmBisome® plus Caspofungin administration. J Pharm Sci 96:1737–1747, 2007
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INTRODUCTION

Fungal infections such as aspergillosis are on the rise in organ transplant recipients, diabetics, and patients with cancer or AIDS. [3,7,10–12,14,18,19,22,28,32,35,45,46] Despite the development of a number of new antifungal agents,[11,22,24,29] amphotericin B (AmpB) formulated as a micelle suspension (i.e., Fungizone®), remains one of the most effective agents in the treatment of systemic fungal infections.[23,29,33,42] However, its use is often limited by the development of dose-dependent renal toxicity.[8,14,23,29,33,44]

To date, a number of chemotherapeutic and antifungal agents have been incorporated into liposomes, resulting in a decrease in the agent's toxicity without loss in their pharmacological effect.[1,4,16,25,26,38,43] In addition, when AmpB was complexed with lipid to form either homemade lipid complexes [37] or AmpB lipid complex (ABLC), it was selectively taken up by mononuclear phagocytes and delivered principally to the liver and the lung.[17,24,25] Survival of mice infected with Histoplasma capsulatum was greater with ABLC than with AmpB treatment, in part due to higher concentrations of AmpB in liver and lung tissue.[37] Moreover, these animals exhibited less toxicity than infected mice administered equivalent amounts of AmpB. Further studies by Bhamra et al. have suggested the very low levels of circulating protein-bound AmpB that they observed after administration of ABLC to rats was a result of rapid tissue uptake leading to reduced toxicity.[5]

AmpB's mechanism of action has been postulated as interacting with the sterol component of fungal cells, ergosterol, which results in pores and channels within the fungal membrane. This results in leaky fungal cell membranes that can cause cell lysis and death. Caspofungin is a new echinocandin in the antifungal group of compounds that inhibits the biosynthesis of beta-(1,3)-D-glucan, an essential component of fungal cell walls and thus inhibits cell wall formation.[10,15,27,39] One study has reported the successful use of Caspofungin as salvage therapy for refractory invasive aspergillosis.[15] The use of Caspofungin with other antifungal agents is appealing due to the drug's distinct mechanism of action. Since both AmpB and Caspofungin distinctly disrupt fungal cell membrane integrity and formation by different mechanisms, it has been hypothesized that a combination of these compounds may enhance their antifungal activity in an additive or synergistic manner without associated toxicity. Although some in vitro [3,10] and clinical [2,9,15,18,39] studies have already reported additive and/or synergistic antifungal activity, to date, few studies have investigated the efficacy and toxicity profile of lipid-based AmpB formulations (i.e., Abelcet® and AmBisome®) when co-administered with Caspofungin. Our group has recently published a study which assessed the antifungal activity of Abelcet® following co-administration of Caspofungin in experimental systemic aspergillosis.[34] To our surprise, this study showed that Abelcet at a dose of 5 mg/kg administered once daily $\times$ 4 days exhibited the greatest antifungal activity compared to the other combination treatment groups tested (i.e., Abelcet + Caspofungin) with no apparent renal and hepatic toxicity.[34] We speculated that this lack of superior antifungal activity from combination therapy may be due to the ability of Caspofungin to modify the pharmacokinetics and reduce the tissue concentrations of AmpB.

In order to investigate this hypothesis, the current study assessed the antifungal activity, pharmacokinetics, and tissue distribution of AmpB following the administration of Abelcet® and AmBisome® in combination with Caspofungin to rats infected with *Aspergillus fumigatus*.

MATERIALS AND METHODS

Study Design

Rats were inoculated with *A. fumigatus* conidia via the indwelling catheter line (jugular vein) 48 h

prior to randomized treatment (Table 1; $n = 6$ for each treatment group). Rats were treated once daily for 4 days and sacrificed on day 5. Organs were harvested at sacrifice (day 5) and processed (see below). Blood was drawn before inoculation (Blank), pre-dose (0 h), 48, and 96 h after treatment. Pharmacokinetic sampling was done 3 days after initial treatment.

Treatment Groups

Rats were randomized into six treatment groups (Table 1). Choice of treatments was made from previous work [34] and others.[21,30] Treatments were prepared according to manufacturer's instructions. Ablecet® was generously donated by Enzon Pharmaceuticals. All other treatments were purchased from Vancouver General Hospital, Department of Pharmacy. Dosing regimens for Abelcet, AmBisome, and Caspofungin used in this study were based on previously published work.[21,30,34]

Animal Model

Male albino Sprague–Dawley rats (350–400 g) were purchased from Charles River Laboratories (Wilmington, MA). The rats were surgically implanted with a port (Access Technologies, Skokie, IL) and catheter with access to venous blood by a similar method used for rabbits.[40] The rats were housed in an animal care facility with a 12-h light-dark cycle and controlled temperature and humidity. The rats were given *ad libitum* access water and standard rat chow (Purina Rat Chow) for the duration of the study. The ports were primed daily with normal saline and heparin to prevent blockages. The animals were cared for according to principles promulgated by the Canadian Council on Animal Care and the University of British Columbia.

Table 1. Treatment Groups in the Study

Treatment	*n*	Dose
Abelcet®[a] (ABLC)	6	5 mg/kg
Ambisome®[b] (liposomal Amphotericin B)	6	5 mg/kg
Cancidas®[c] (Caspofungin)	6	3 mg/kg
Abelcet®[a] + Cancidas®[c]	6	5 mg/kg; 3 mg/kg
Ambisome®[b] + Cancidas®[c]	6	5 mg/kg; 3 mg/kg
Normal saline (control)	6	

Prior to 24, 48, and 144 h following Abelcet, AmBisome, or NS administration, serum samples were obtained for serum creatinine measurements as an indirect evaluation of renal function and for serum AST, measurements as an indirect evaluation of hepatic function. Following the 144 h blood collection, each rat was sacrificed by injecting a single intraperitoneal dose of sodium pentobarbital (300 mg/kg); the kidneys, spleen, lung, liver, heart, and brain were removed, blotted dry, and weighed. The degree of antifungal activity was determined by measuring the number of colony forming units (CFU) of *Aspergillus fumigatus* in tissues.

[a]Enzon Pharmaceuticals.

[b]Astellas Pharma/Gilead Sciences.

[c]Merck & C.

Aspergillus fumigatus Inoculum

Modified from previously published work,[34] *A. fumigatus* was collected from a patient with disseminated aspergillosis (BC Centre for Disease Control, F1048). Cultures were grown on Sabouraud dextrose agar for 48 h at 37°C. Conidia were isolated by washing the agar with pyrogen-free saline. The conidia were suspended by vortexing with glass beads and diluted with pyrogen-free saline to obtain between 2.1 and 2.5×10^7 conidia in 300 μL of saline. Conidia were counted using a hemocytometer and a 100 μL aliquot was serially diluted and aliquots were plated on sabouraud dextrose agar for 48 h at 37°C to determine the number of viable conidia and purity of the inoculum. The average percentage of viable conidia in the inoculum was $62\% \pm 19$. None of the spore suspensions were contaminated with any other organism. Rats were inoculated with 300 μL through the indwelling port 48 h before the beginning of treatment to allow aspergillosis to develop.

Sample Processing

One-milliliter whole blood samples were drawn into pediatric collection tubes (3.6 mg K_2 EDTA) before infection (blank), pre-dose (0 h), 48 h after first treatment (48 h), and 96 h after first treatment (96 h). Whole blood (300 μL) was drawn for pharmacokinetic analysis on day 4 of treatment at pre-dose (0 h), 5 min, 10 min, 20 min, 30 min, 4 h, 8 h, and 24 h after treatment. All whole blood samples were mixed by inversion and plasma was separated by centrifugation (15 min, 3000 rpm at 4°C). Plasma samples were stored at −20°C until HPLC analysis.

After the collection of the 96-h blood specimen, the rat was euthanized with intravenous overdose (1 mL) of Euthanyl®, (sodium pentobarbital 240 mg/mL). Spleen, right kidney, liver, lung, heart, and brain tissue samples were harvested,

weighed, and placed in sterile containers. Normal saline was added to 1 mL/g of specimen and homogenized (Heidolph diax 900). An aliquot of organ homogenate was stored at room temperature until plating and the remaining sample was placed at −80°C until HPLC analysis.

Amphotericin B Plasma and Organ Determinations

For plasma determinations, 150 μL of standards, quality control samples (QCs), and unknown were aliquoted. Proteins were precipitated with HPLC grade methanol (Fisher Scientific, Ottawa, ON, Canada) and removed by centrifugation. The supernatant was removed and evaporated to dryness with nitrogen gas in a 50°C water bath. The residue was reconstituted with HPLC grade methanol and 30 μL was injected.

For organ determinations, 150 μL of standards, QCs, and unknown organ homogenate were aliquoted. Proteins were precipitated with HPLC grade methanol and removed by centrifugation. Supernatant (20 μL) was injected.

The analytical method was a modified method previously described.[41] Briefly, the method uses a 2.0×150 mm, 5 μm particle, C18 analytical column (Phenomenex Luna®) with matching guard cartridge (Phenomenex Security Guard®). A 12-min gradient run time (Mobile Phase A = 10 mM Sodium Acetate; B = HPLC grade Acetonitrile) at 0.35 mL/min was used to separate AmpB from matrix components on a Waters® 2695 Separation Unit with UV detection ($\lambda = 408$) on a Waters® 2996 PDA. Unknown sample peak area was compared to an extracted standard curve. QCs were used to confirm predictability of the standard curve and assay robustness.

Antifungal Activity

The choice of organ colony forming units (CFU) as an indicator of antifungal activity was based on previously published work.[34] Plasma galactomannan antigen detection was added based on the work published by Marr et al. [20] which showed a correlation between tissue fungal burden and galactomannan antigen index value.

Organ CFU Determinations

Aliquots of 100 μL full strength organ homogenate and 1:10 dilution (with sterile saline) were each spread plated onto Saboraud Dextrose Agar plates in duplicate. After 48 h incubation at 37°C, the resulting colonies of *A. fumigatus* were counted and averaged over the duplicate plates. The limit of detection of the assay was 0.1×10^2 CFU/mL homogenate.

Plasma Galactomannan Determinations

Using a commercially available sandwich ELISA kit (Platelia Aspergillus, Bio-Rad Laboratories, Montreal, QC, Canada), rat plasma samples were processed as per manufacturer's instructions. Briefly, 300 μL of negative control, positive control, calibrator, and unknown sample plasma (Blank, 0, 48, and 96 h samples) were treated, incubated at 100°C for 3 min, and centrifuged (10 min @ 10000*g*, room temperature). Conjugate (50 μL) was added to the wells containing the antibodies to galactomannan. Sample supernatant (50 μL) was added to the wells and incubated for 90 min at 37°C. The wells were then washed (five times with diluted washing agent). Substrate buffer (200 μL) was added to each well and incubated in the dark for 30 min. The reaction was stopped with 100 μL 1.5 M sulfuric acid. Optical density was measured at 450 nm. Unknowns were compared to the calibrator (1 ng/mL) and an index value was calculated ($OD_{unkown}/OD_{Calibrator}$) and reported. An index value greater than 1 was considered to be positive for galactomannan antigen.

Renal Toxicity

Renal toxicity was indirectly assessed, as previously described,[34] by determining creatinine concentration in plasma using a commercially available kit (Sigma Chemicals Co., St. Louis, MO). A baseline was determined by measuring creatinine concentration in the Blank sample, and was compared to plasma creatinine concentration in the 0 (pre-dose), 48, and 96 h samples. For the purposes of this study, a 50% or greater increase in plasma creatinine concentration as compared to baseline was considered to be a sign of renal toxicity.

Pharmacokinetic and Statistical Analysis

The pharmacokinetic parameters, mean residence time (MRT), total body clearance (CL), and volume of distribution during the terminal (V_d), were estimated by non-compartmental analysis using the WINNONLIN Professional (v5.0.1; Pharsight) non-linear estimation program.[44] Concentrations of AmpB in plasma were plotted against time on log-linear graph paper and

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terminal half-life was estimated by method of residuals.[44] Area under the AmpB concentration-time curve (AUC_{0-24}) was estimated by trapezoidal rule.[44] Final parameters were compared (Abelcet to Abelcet + Caspofungin and Ambisome to Ambisome + Caspofungin) using an unpaired *t*-test (Prism 4; Graphpad, Inc., San Diego, CA). The number of CFUs in organs, plasma creatinine concentrations prior to and following administration of treatment were compared between each treatment group by analysis of variance (INSTAT2; GraphPad, Inc.). Critical differences were assessed by Tukey *post hoc* tests. Galactomannan Index Values were compared prior to 48 and 96 h following treatment using repeat measures ANOVA with a Tukey *post hoc* test to determine critical differences (Prism 4; Graphpad, Inc.). AmpB tissue concentrations (Abelcet to Abelcet + Caspofungin and Ambisome to Ambisome + Caspofungin) using an unpaired *t*-test (Prism 4; Graphpad, Inc.). A difference was considered significant if the probability of chance explaining the results was reduced to less than 5% ($p < 0.05$).[6] All data were expressed as a mean ± standard error of the mean.

RESULTS

Abelcet® treatment significantly decreased total fungal CFU concentrations recovered in all the organs added together by 73% compared to non-treated controls (1845 ± 605 vs. 6890 ± 3484 CFU/mL of homogenized tissue; $p < 0.05$) (Table 2). Ambisome® treatment significantly decreased total fungal CFU concentrations recovered in all the organs added together by 69% compared to non-treated controls (2128 ± 969 vs. 6890 ± 3484 CFU/mL of homogenized tissue; $p < 0.05$) (Table 2). Caspofungin® treatment significantly decreased total fungal CFU concentrations recovered in all the organs added together by 80% compared to non-treated controls (1406 ± 949 vs. 6890 ± 3484 CFU/mL of homogenized tissue; $p < 0.05$) (Table 2). Abelcet® plus Caspofungin® treatment significantly decreased total fungal CFU concentrations recovered in all the organs added together by 81% compared to non-treated controls (1285 ± 643 vs. 6890 ± 3484 CFU/mL of homogenized tissue; $p < 0.05$) (Table 2). Ambisome® plus Caspofungin® treatment significantly decreased total fungal CFU concentrations recovered in all the organs added together by 98% compared to non-treated controls (175 ± 43 vs.

Table 2. Fungal Analysis of *Aspergillus fumigatus* Infected Male Sprague–Dawley Rats Treated with Single Intravenous Doses of Normal Saline (Non-Treated Control), ABLC (5 mg/kg × 4 Days), AmBisome (5 mg/kg × 4 Days), Caspofungin (3 mg/kg × 4 Days), Abelcet (5 mg/kg × 4 Days) + Caspofungin (3 mg/kg × 4 days), and AmBisome (5 mg/kg × 4 Days) + Caspofungin (3 mg/kg × 4 Days)

	Infected Tissues (CFU/mL of Homogenized Tissues)						
Treatment Groups	Brain	Lungs	Heart	Liver	Spleen	Kidney	All Organs
Non-treated controls ($n = 6$)	3987±1986	40±20	5±3	275±251	1983±1808	92±90	6890±3484
Single drug therapy							
Abelcet 5 ($n = 6$)	1777±605	3±2[a]	15±13	18±9	10±5[a]	22±8	1845±605[a]
AmBisome 5 ($n = 6$)	2053±956	10±6[a]	17±9	ND	25±9[a]	23±16	2128±969[a]
Caspofungin 3 ($n = 5$)	738±452[a]	6±3[a]	28±23	50±27	350±286	234±227	1406±949[a]
Combination therapy							
Abelcet 5 + Caspofungin 3 ($n = 6$)	1165±608[a]	3±2[a]	48±44	20±12	32±26[a]	17±8	1285±643[a]
Ambisome 5 + Caspofungin 3 ($n = 6$)	1 30±47[a,b,c,d,]	7±5[a]	12±4	3±2	13±7[a]	10±4	175±43[a,b,c,e]

All rats were infected with $2.1–2.5 \times 10^7$ viable colony forming units (CFU)/0.3 mL/rat of *Aspergillus fumigatus* prior to initiation of treatment.
[a] $p < 0.05$ versus non-treated controls.
[b] $p < 0.05$ versus Abelcet 5 mg/kg alone.
[c] $p < 0.05$ versus Ambisome 5 mg/kg alone.
[d] $p < 0.05$ versus Caspofungin alone.
[e] $p < 0.05$ versus Abelcet 5 + Caspofungin 3 using PCANOVA; all data are presented as mean ± SEM. ND, below the detectable limit of the CFU assay.

6890 ± 3484 CFU/mL of homogenized tissue; $p < 0.05$) (Table 2).

To confirm these findings, a second method for assessing anti-fungal activity, plasma galactomannan levels, was determined (Figure 1). Abelcet® treatment significantly decreased plasma galactomannan levels by 50 and 75% 96 h following the initiation of treatment in the absence and presence of Caspofungin co-therapy respectively (Figure 1A and B). AmBisome® treatment significantly decreased plasma galactomannan levels by 73 and 78% 96 h following the initiation of treatment in the absence and presence of Caspofungin co-therapy, respectively (Figure 1C and D). Plasma galactomannan levels following Caspofungin treatment alone were not reported because it has been previously reported [15,20] and confirmed in this study (data not shown) that administration of Caspofungin does not significantly alter plasma galactomannan levels and thus is not an accurate marker of Caspofungin antifungal activity.

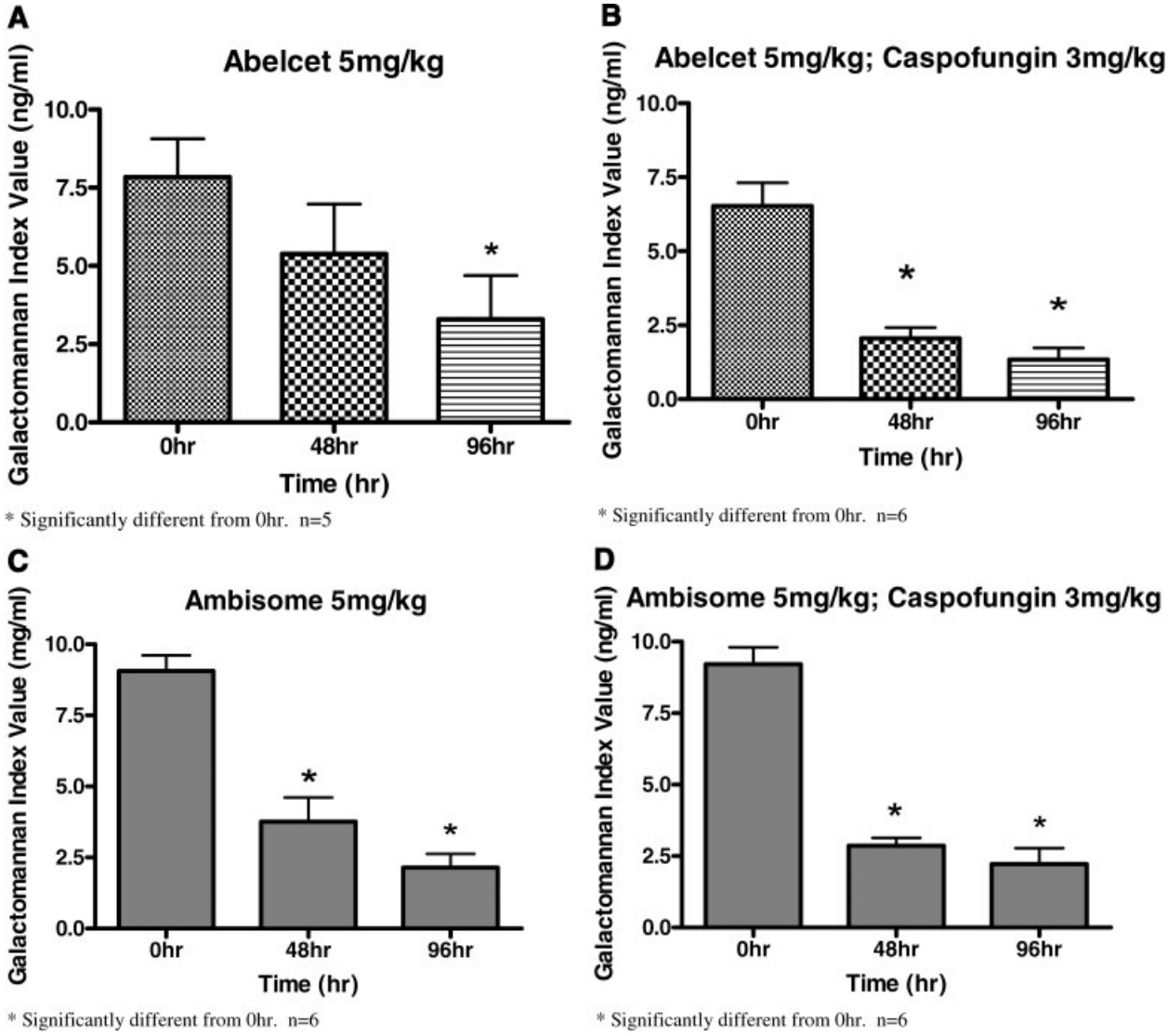

Figure 1. Plasma galactomannan concentrations of *Aspergillus fumigatus* infected Male Sprague–Dawley Rats treated with single intravenous doses (A) Abelcet (ABLC) (5 mg/kg × 4 days), (B) Abelcet (ABLC) (5 mg/kg × 4 days) + Caspofungin (3 mg/kg × 4 days), (C) AmBisome (5 mg/kg × 4 days), Caspofungin (3 mg/kg × 4 days), and (D) AmBisome (5 mg/kg × 4 days) + Caspofungin (3 mg/kg × 4 days). All rats were infected with $2.1–2.5 \times 10^7$ Viable Colony Forming Units (CFU)/0.3 mL/rat of *Aspergillus fumigatus* prior to initiation of treatment.

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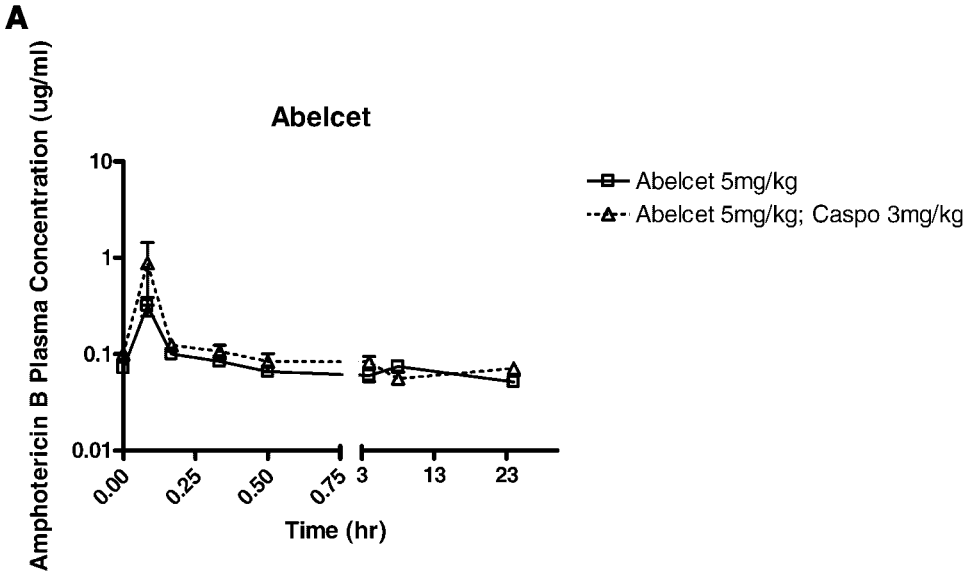
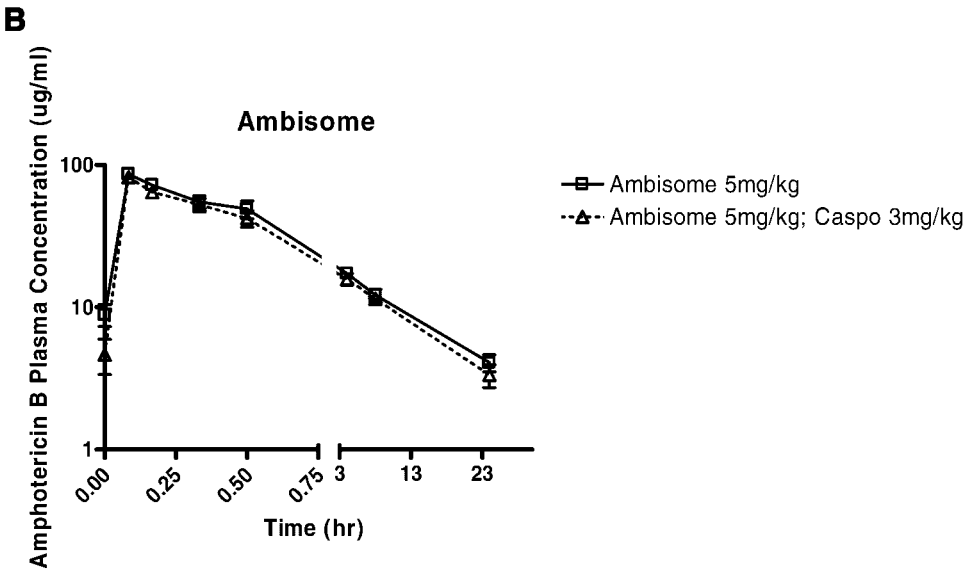
Plasma concentration versus time course profile is reported in Figure 2. No significant differences in AmpB plasma concentration versus time were observed following the administration of Abelcet or Ambisome in the presence or absence of Caspofungin therapy. Pharmacokinetic analysis for AmBisome is reported in Table 3. No significant differences in AmpB terminal half-life, area-under-plasma concentration curve, volume of distribution in the terminal phase, plasma clearance, and mean residence time were observed following the administration of AmBisome in the



Data are presented as mean ±standard error of the mean. Abelcet n=4, Abelcet ; Caspo n=5



Data are presented as mean ± standard error of the mean. Ambisome n=6, Ambisome; Caspo n=6

Figure 2. AmpB plasma concentration versus time profiles for *Aspergillus fumigatus* infected Male Sprague–Dawley Rats treated with single intravenous doses of (A) Abelcet (ABLC) (5 mg/kg × 4 days), Abelcet (ABLC) (5 mg/kg × 4 days) + Caspofungin (3 mg/kg × 4 days) (B) AmBisome (5 mg/kg × 4 days), Caspofungin (3 mg/kg × 4 days), and AmBisome (5 mg/kg × 4 days) + Caspofungin (3 mg/kg × 4 days). All rats were infected with $2.1–2.5 \times 10^7$ Viable Colony Forming Units (CFU)/0.3 mL/rat of *Aspergillus fumigatus* prior to initiation of treatment. Measurements were from day 3.

presence or absence of Caspofungin therapy. For Abelcet and Abelcet + Caspofungin, the AmpB plasma concentration profile produced by the time points drawn did not allow for a comprehensive analysis of pharmacokinetic parameters because a terminal phase could not be identified in all rats in the two groups. There appeared to be a redistribution/reabsorption phase between the 4 and 24 h time points. A similar result was observed in the work of Bhamra et al.[5], with an increase in AmpB plasma concentration at the 12 h time point before the terminal phase. The AUC_{0-24} was calculated for the Abelcet group (1.5 ± 0.2 $N = 4$) and compared to the AUC_{0-24} for the Abelcet + Caspofungin group (1.7 ± 0.2 $N = 5$). The difference was compared in an unpaired t-test and was found to be not significant. No significant differences in AmpB tissue concentrations were observed following the administration of Abelcet or Ambisome in the presence or absence of Caspofungin therapy (Table 4). No significant differences in plasma serum creatinine concentrations prior to and follow therapy were observed for all treatment groups tested (data not shown).

DISCUSSION

The use of Caspofungin as salvage therapy for invasive aspergillosis has resulted in a response rate of 40–50% among a variety of patient populations. The use of Caspofungin with other antifungal agents is appealing due to the drug's distinct mechanism of action. Since both AmpB and Caspofungin distinctly disrupt fungal cell membrane integrity and formation by different mechanisms, it has been hypothesized that a combination of these compounds may enhance their antifungal activity in an additive or synergistic manner without associated toxicity. The purpose of this study was to assess the antifungal activity, pharmacokinetics, and tissue distribution of AmpB following the administration of Abelcet® and AmBisome® alone and in combination with Caspofungin® to rats infected with *Aspergillus fumigatus*.

Previous studies, primarily within patients, have reported the benefits of caspofungin in combination with liposomal amphotericin (and not Abelcet) as primary or salvage treatment of invasive aspergillosis in cancer[2,15] and pediatric patients.[9] In addition, we have recently reported that Abelcet in combination with Caspofungin was not significantly more effective than either Abelcet or Caspofungin alone in decreasing organ CFU concentrations. An explanation for this lack of superior antifungal activity from Abelcet plus Caspofungin combination therapy was the subject of this study.

Similar to our findings reported in our previous article[34], no significant differences in antifungal activity (Table 2) were observed between Abelcet at 5 mg/kg × 4 days, Caspofungin at 3 mg/kg × 4 days, and the combination of Abelcet and Caspofungin (Table 2). However, the combination of AmBisome plus Caspofungin exhibited significantly greater reduction in organ CFU concentrations than any of the other treatment groups tested (Table 2). We speculated that these observations may be due to the ability of Caspofungin to modify the pharmacokinetics and tissue distribution of AmpB following co-administration with these lipid-based AmpB formulations. However, we found co-administration of Caspofungin with Abelect and AmBisome did not significantly alter the plasma concentration-time profile (Figure 2), pharmacokinetic parameters (Table 3 for AmBisome only) and tissue distribution (Table 4) of

Table 3. Amphotericin B (AmpB) Pharmacokinetic Analysis from Male Sprague–Dawley Rats Infected with *Aspergillus fumigatus* Treated with Single Intravenous Doses of AmBisome (5 mg/kg × 4 Days) and AmBisome (5 mg/kg × 4 Days) + Caspofungin (3 mg/kg × 4 Days)

Treatment	$t_{1/2}$ (h)	AUC_{0-24} (h·μg/mL)	V_d (mL)	Cl (mL/h)	MRT (h)
Ambisome 5 mg/kg	9.8 ± 0.8 $N = 6$	334.0 ± 33.9 $N = 6$	67.3 ± 8.4 $N = 6$	4.9 ± 0.8 $N = 6$	6.2 ± 0.2 $N = 6$
Ambisome 5 mg/kg; Caspofungin 3 mg/kg	9.0 ± 0.8 $N = 6$	301.4 ± 26.6 $N = 6$	70.3 ± 7.6 $N = 6$	5.6 ± 0.6 $N = 6$	6.1 ± 0.1 $N = 6$

No parameters are significantly different (unpaired t-test). Data are mean ± standard error of the mean.

$t_{1/2}$, half-life; AUC, area under the concentration time curve for 0–24 h; Vd, Volume of distribution in terminal phase; Cl, AmpB plasma clearance; MRT, AmpB mean residence time.

For ABLC and ABLC + Caspofungin, the amphotericin B plasma concentration profile produced by the time points drawn did not allow for a comprehensive analysis of pharmacokinetic parameters because a terminal phase could not be identified in all rats in the two groups.

Table 4. Amphotericin B (AmpB) Tissue Distribution in Male Sprague–Dawley Rats Infected with *Aspergillus fumigatus* Treated with Single Intravenous Doses of Abelcet (5 mg/kg × 4 days), Abelcet (5 mg/kg × 4 Days) + Caspofungin (3 mg/kg × 4 Days), AmBisome (5 mg/kg × 4 Days), and AmBisome (5 mg/kg × 4 Days) + Caspofungin (3 mg/kg × 4 Days)

Treatment	Amphotericin B Tissue Concentration (μg/g)					
	Brain	Liver	Heart	Lung	Kidney	Spleen
Abelcet 5 mg/kg	N/A	$35.3 \pm 5.3\ N = 6$	$1.0 \pm 0.1\ N = 6$	$9.3 \pm 1.2\ N = 6$	$1.4 \pm 0.1\ N = 6$	$64.6 \pm 10.4\ N = 6$
Abelcet 5 mg/kg; Caspofungin 3 mg/kg	N/A	$38.0 \pm 3.5\ N = 6$	$0.9 \pm 0.2\ N = 6$	$9.552 \pm 2.363\ N = 6$	$1.5 \pm 0.3\ N = 6$	$53.5 \pm 11.0\ N = 6$
Ambisome 5 mg/kg	$0.7 \pm 0.1\ N = 5$	$110.2 \pm 6.5\ N = 6$	$0.6 \pm 0.1\ N = 6$	$2.6 \pm 0.9\ N = 6$	$1.1 \pm 0.1\ N = 6$	$17.5 \pm 2.6\ N = 6$
Ambisome 5 mg/kg; Caspofungin 3 mg/kg	$0.5 \pm 0.04\ N = 3$	$106.5 \pm 8.0\ N = 6$	$0.6 \pm 0.1\ N = 6$	$3.3 \pm 0.7\ N = 6$	$1.4 \pm 0.2\ N = 6$	$9.5^{*} \pm 0.9\ N = 6$

*Significantly different (unpaired *t*-test) $p < 0.05$. Data are mean ± standard error of the mean.
Data are mean ± standard error of the mean. All values for brain were below the quantitative limit of the assay and therefore, could not be compared (N/A).
$p < 0.05$ versus Ambisome alone.

AmpB. Taken together, these findings suggest that Caspofungin may alter AmBisome but not Abelcet's antifungal activity at a cellular level rather than by altering AmpB's disposition. Recently, Ripeau et al. have reported that Caspofungin can increase the expression of *Candida albicans* secretory proteinase 5.[31] Secretory proteinases may selectively release active AmpB from Ambisome but not Abelcet at sites of fungal infection in a similar way as fungal cell-derived phospholipases [36] resulting in more active AmpB available to cause damage to fungal cells. Studies to test this hypothesis are on-going. An additional hypothesis to consider is the effect of these AmpB lipid-based formulations on the pharmacokinetics and tissue distribution of Caspofungin.[13] Such investigations are warranted in the future.

In conclusion, our findings suggest that an alternative mechanism possibly at the cellular level rather than altered AmpB disposition may be an explanation for the differences in organ CFU concentrations following Abelcet® plus Caspofungin versus AmBisome® plus Caspofungin administration.

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