Pharmacodynamics of Liposomal Amphotericin B and Flucytosine for Cryptococcal Meningoencephalitis: Safe and Effective Regimens for Immunocompromised Patients

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Background. Cryptococcal meningoencephalitis is a lethal infection with relatively few therapeutic options. The optimal dosage of liposomal amphotericin B (LAmB) alone or in combination with flucytosine is not known.

Methods. A murine model of cryptococcal meningoencephalitis was used. The fungal density in the brain was determined using quantitative cultures. Pharmacokinetic–pharmacodynamic relationships were determined for LAmB and flucytosine administered alone. The effect of the combination was described using the Greco model and a mathematical model. The results were bridged to humans.

Results. Inoculation resulted in hematogenous dissemination and logarithmic growth within the central nervous system. There was histological evidence of multifocal infection throughout the brain. Both LAmB and flucytosine produced a dose-dependent reduction in fungal burden. The effect of the combination in the brain was additive. Bridging studies suggested that a human dosage of LAmB 3 mg/kg/d resulted in a submaximal antifungal effect. Regimens of LAmB 6 mg/kg/d alone, LAmB 3 mg/kg/d plus flucytosine 50 mg/kg/d, and LAmB 3 mg/kg/d plus flucytosine 100 mg/kg/d all resulted in near-maximal antifungal activity.

Conclusions. Potential regimens for further study in clinical trials include LAmB 6 mg/kg/d alone, LAmB 3 mg/kg/d plus flucytosine 50 mg/kg/d, and LAmB 3 mg/kg/d plus flucytosine 100 mg/kg/d.

Keywords. Cryptococcus; meningitis; meningoencephalitis; liposomal amphotericin B; flucytosine.
vs 6 mg/kg/d vs amphotericin B deoxycholate showed no differences between any of these regimens [4]. Conversely, a small study using 4 mg/kg/d of LAmB resulted in a significantly earlier cerebrospinal fluid (CSF) culture conversion than treatment with 0.7 mg/kg/d of amphotericin B deoxycholate [5]. Therefore, there is no known optimal initial dosage of a lipid formulation for cryptococcal meningoencephalitis. Furthermore, the potential benefit of flucytosine for patients with cryptococcal meningoencephalitis is not known nor is the smallest effective dose that can be used in combination with LAmB [6].

Here, we use a previously described murine model of cryptococcal meningoencephalitis [7] to define the pharmacokinetics and pharmacodynamics of LAmB and flucytosine alone and in combination. We bridged the results to humans in order to provide further insight into combination regimens that are likely to be safe and effective. These results can now be used to aid in the design of clinical trials to further understand the optimal use of these compounds in humans with cryptococcal meningoencephalitis.

METHODS

Organism
A well-characterized clinical isolate of *Cryptococcus neoformans* var. *grubii*, H99 (American Type Culture Collection [ATCC 208821]), was used (LGC Standards). The isolate was stored on beads at −80°C. Seventy-two hours prior to inoculation, the organism was plated to Sabouraud dextrose agar (Oxoid, Basingstoke, UK) containing chloramphenicol (Sigma-Aldrich Company Ltd., UK) and incubated at 30°C for 48 hours. Twenty-four hours prior to inoculation, several colonies were placed into 10 mL of Sabouraud dextrose broth (Oxoid) and incubated at 37°C on a shaker. Immediately prior to inoculation, the fungal suspension was centrifuged and washed 3 times with phosphate buffered saline (PBS; GIBCO, Invitrogen, Paisley, UK). The fungal inoculum was prepared by resuspension of the stock solution with 5% glucose (Baxter, Thetford, UK). The final fungal inoculum was prepared by resuspension and dilution in PBS. A hemocytometer was used to achieve the desired concentration of 3.8 × 10^5 CFU/mL.

Minimum Inhibitory Concentrations
The in vitro susceptibility of H99 to amphotericin B and flucytosine was determined using Clinical and Laboratory Standards Institute methodology (M27-A3) [8]. The minimum inhibitory concentration (MIC) of each agent was determined over the course of 5 independently conducted experiments.

Antimicrobial Agents
LAmB for injection was reconstituted according to the manufacturer’s instructions. The desired concentrations were obtained by dilution of the stock solution with 5% glucose (Baxter, Thetford, UK). The intravenous clinical preparation of flucytosine (Valeant Pharmaceuticals Ltd., Basingstoke, UK) was used for all murine experiments but administered orally.

Murine Model of Cryptococcal Meningoencephalitis
A previously described model of cryptococcal meningoencephalitis was used [7]. Male CD1 mice (Charles River Ltd, Kent, UK) were housed in groups of 5. Mice were inoculated via a lateral tail vein with 9.5 × 10^4 cryptococcal organisms in a 0.25-mL suspension.

Histology and Immunohistochemistry
Brains were fixed in 10% neutral buffered formalin, cryoprotected, and embedded in OCT freezing medium (Tissue–Tek® O.C.T. Compound); 5-µm-thick sections were then cut. Tissue sections were treated with peroxidase solution (Envison+ kit, Dakocytomation, Carpinteria, California) to block endogenous peroxidase activity. A 1.5% goat serum protein block was applied to reduce nonspecific binding. Sections were incubated with an anti-*Cryptococcus* monoclonal antibody (Mybiosource, San Diego, California; 1:1000 dilution). Slides were reacted with a peroxidase-labeled anti-mouse polymer (Envison+ kit, Dakocytomation). Antibody–antigen complexes were visualized using 3′-diaminobenzidine chromogen substrate (Dakocytomation) for the peroxidase reaction. Slides were counterstained with hematoxylin (Richard-Allan Scientific, Kalamazoo, Michigan).

![Figure 1](https://example.com/figure1.png)

**Figure 1.** The rate of change of fungal density in the cerebrum of mice infected with *Cryptococcus neoformans* at time zero. Data are pooled from 5 experiments and are mean ± SD of 3–4 mice. The solid line is the fit of the model dN/dt = Kgmax*(N/POPMAX)*N to the data, where N is the number of organisms, Kgmax is the growth constant, and POPMAX is the maximal fungal density in the brain. The estimates for the parameter values are Kgmax = 0.08 log_10 CFU/g brain/h and POPMAX = 6.41 log_10 CFU/g brain and the number of organisms in the brain immediately after inoculation of 2.00 log_10 CFU/g brain; r^2 = 0.88. The corresponding histological sections stained with hematoxylin and eosin and anti–*C. neoformans* antibody and taken on days 1, 3, 5, and 7 post infection are shown. A high power section shows the formation of cyst-like structures with leaching of cryptococcal capsular material into the contiguous parenchyma.
Pharmacokinetics and Pharmacodynamics of LAmB and Flucytosine as Monotherapy

Therapy with LAmB and flucytosine commenced 24 hours post inoculation. Informative parts of the exposure–response relationships were defined in preliminary dose-finding experiments.

Dosages of LAmB 3, 10, and 20 mg/kg were administered every 24 hours via a lateral tail vein in a volume of 0.25 mL. The respective controls received 0.25 mL of 0.9% sodium chloride once daily. The intravenous formulation of flucytosine 25, 50, and 100 mg/kg (ie, 75, 150, and 300 mg/kg/d) was administered.

**Figure 2.** The pharmacokinetics of liposomal amphotericin B (LAmB) 3, 10, and 20 mg/kg/d intravenously in plasma and cerebrum. Data are mean ± SD, and the solid line is the fit of the model to the data.
every 8 hours by oral gavage in a volume of 0.25 mL. Control mice received 0.25 mL of the vehicle, 0.9% sodium chloride, every 8 hours by oral gavage.

The concentration–time profiles of LAmB in plasma and cerebrum were defined using cohorts of mice (n = 3 for each dose–timepoint combination). Mice were sacrificed at 1, 2, 4, 6, and 24 hours post dose. Plasma was obtained by cardiac puncture and stored at −80°C. The brain was removed, placed in a sterile plastic bag, and stored at −80°C. A similar approach was used for flucytosine except a different sampling strategy was used (plasma and cerebrum samples were obtained 0.5, 1, 3, and 8 hours post dose).

The temporal change in cerebral fungal burden following treatment with both agents was defined using a serial sacrifice design. Cohorts of mice (n = 3 for each regimen at each timepoint) were sacrificed at 1, 24, 72, 120, and 168 hours post infection. The pharmacodynamic relationships were defined over 7 experiments. The entire brain was removed and homogenized in 1 mL PBS; serial 10-fold dilutions were plated to enumerate the fungal density in cerebrum.

**Pharmacodynamics of LAmB and Flucytosine in Combination**

The pharmacodynamics of the combination of LAmB and flucytosine were defined in a single experiment with 116 mice. The combination experiment was designed following monotherapy experiments. Cohorts of mice (n = 12) received the following dosage regimens: LAmB 3 mg/kg/d alone; LAmB 20 mg/kg/d alone; flucytosine 25 mg/kg every 8 hours (ie, 75 mg/kg/d) alone; flucytosine 100 mg/kg every 8 hours (ie, 300 mg/kg/d) alone; LAmB 3 mg/kg/d with flucytosine 25 mg/kg every 8 hours; LAmB 3 mg/kg/d in combination with flucytosine 100 mg/kg every 8 hours; LAmB 20 mg/kg/d in combination with flucytosine 25 mg/kg every 8 hours; and LAmB 20 mg/kg in combination with flucytosine 100 mg/kg every 8 hours. Mice in the control group received 0.25 mL of the vehicle, 0.9% sodium chloride every 8 hours by oral gavage. Cohorts of mice (n = 4 for each regimen at each timepoint) were sacrificed at 72, 120, and 168 hours post infection. In addition, 2 groups of control mice (n = 4) were sacrificed at 1 and 24 hours post inoculation. Plasma samples were collected by cardiac puncture (as above) after terminal anesthesia with 5% isoflurane. Mice were then sacrificed by cervical dislocation, and whole brains were excised aseptically for quantitative culture (as above). Sampling times were chosen to obtain trough concentrations of each antimicrobial agent in plasma and cerebrum samples immediately prior to dosing on the second, fourth, and sixth days post initiation of therapy in the murine model. Trough concentrations from combination therapy were compared with data from the monotherapy experiments to examine the possibility of a pharmacokinetic interaction.

**Measurement of Antifungal Drug Concentrations in Plasma and Cerebrum**

The total concentrations of amphotericin B and flucytosine in plasma and cerebrum samples were measured by high-performance liquid chromatography (Shimadzu Prominence; Shimadzu, Milton Keynes, UK). For amphotericin B, the internal standard was piroxicam (Sigma-Aldrich Company Ltd.). A standard curve was constructed over the concentration range 0.05–100 mg/L. Plasma and cerebrum homogenate were mixed with 300 µL methanol containing the internal standard, and 50 µL was injected onto a 5-µm 50 × 20 mm column (Varian Pursuit C18, Varian Inc., UK). The mobile phase consisted of a starting concentration of 80% of 0.1% aqueous formic acid (v/v; Fisher Scientific, Loughborough, UK) and 20% of 0.1% formic acid in acetonitrile (v/v; Fisher Scientific), progressing with a gradient to a 30:70 mix over 7 minutes. The run time was 9.5 minutes, with a flow rate of 0.8 mL/min. Amphotericin B and the internal standard eluted after 4.0 minutes and 2.3 minutes, respectively, and were detected using ultraviolet absorbance at 385 nm. The coefficient of variation was <6%. The limit of detection was 0.05 mg/L, and the intra- and interday variations for the method were <8%.

For flucytosine, the internal standard was chlorouracil (Sigma-Aldrich Company Ltd.). A standard curve encompassing 0.39–400 mg/L was constructed. Plasma and cerebrum homogenate were mixed with only 10 µL of internal standard solution, but an additional 200 µL trichloracetic acid (Fisher Scientific) at 30 mg/mL was added. Samples were then centrifuged for 10 minutes before 200 µL of supernatant was removed. Then 20 µL of the extracted sample was injected onto a Luna 5 µm C18 (2) 100A 250 × 4.6 mm column (Phenomenex, Macclesfield, UK). The mobile phase used starting concentrations of 100% of 10 mM potassium dihydrogen orthophosphate (Fisher Scientific) and 0% of 0.1% formic acid in acetonitrile (Fisher Scientific), progressing to 60% and 40%, respectively, with a gradient over 5 minutes. The overall run time was 11 minutes, with a flow rate of 1 mL/min. Flucytosine and the internal standard eluted after 6.1 minutes and 9.5 minutes, respectively, and were detected using ultraviolet absorbance at 266 nm. The coefficient of variation percentage was <7%, the limit of detection was 0.39 mg/L, and the intra- and interday variations were <7%.

**Modeling of Drug Combination and Mathematical Modeling**

The antifungal effect of the combination of LAmB and flucytosine was modeled using the Greco model implemented within ADAPT 5, as previously described [9]. For this model, only data from a single point in time at the end of the experiment were used (ie, 168 hours post inoculation). The Greco model takes the form:

\[
1 = \frac{D_{LAmB}}{E_{50,LAmB} \cdot [E/Econ - E]^{1/m,LAmB}} + \frac{D_{5FC}}{E_{50,5FC} \cdot [E/Econ - E]^{1/m,5FC}} + \frac{\alpha \cdot D_{LAmB} \cdot D_{5FC}}{E_{50,LAmB} \cdot E_{50,5FC} \cdot [E/Econ - E]^{(1/2m,LAmB+1/2m,5FC)}}
\]
where $D_{LAmB}$ and $D_{SFC}$ are the dosages of LAmB and flucytosine producing the antifungal effect, $E_{i}$: Econ is the fungal density in untreated mice; $E_{S0,LAmB}$ and $E_{S0,SFC}$ are the total daily dosages producing 50% of the maximal effect of LAmB and flucytosine, respectively; $m_{LAmB}$ and $m_{SFC}$ are the respective slope parameters for each agent; and $\alpha$ is the interaction parameter. The first 2 terms define the additive effect, while the third is the interaction term, containing the interaction parameter $\alpha$. If $\alpha = 0$, the combination is additive (because the third term is zero). If $\alpha$ is positive and its lower 95% confidence bound does not cross zero, synergy is present. If $\alpha$ is negative and its upper 95% confidence bound does not cross zero, antagonism is present.

A mathematical model was fitted to the entire dataset in order to describe the following: the link between plasma and cerebral concentrations of LAmB and flucytosine and (2) the relationship between cerebral concentrations of both antifungal agents and the antifungal effect in the brain when administered alone and in combination. The mathematical model allowed definition of the antimicrobial drug exposure that produced near-maximal antifungal activity. The model was fit to the data using a population methodology with the program Big Non-Parametric Adaptive grid [10]. The mathematical model (see Supplementary Appendix) incorporated a unique interaction model in which the concentrations of flucytosine and LAmB were allowed to affect the magnitude of the rate of maximum antifungal killing attributable to the other drug. The weighting terms for the pharmacokinetic–pharmacodynamic data were determined using the maximum-likelihood estimator in the program ADAPT 5 [11], as previously described [12].

**RESULTS**

**Minimum Inhibitory Concentrations**

The modal MICs for LAmB and flucytosine were 1 mg/L and 0.125 mg/L, respectively.

**Murine Model of Cryptococcal Meningoencephalitis**

Inoculation resulted in reproducible hematogenous dissemination of *C. neoformans* to the brain. There was progressive logarithmic growth in cerebral fungal burden throughout the experimental period (Figure 1). Consistent with other disseminated models with H99 (eg, [15]), there was no mortality in

**Table 1. Estimates for the Parameter Values From the Mathematical Model**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
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<tbody>
<tr>
<td>$K_{Sf}$ (h$^{-1}$)</td>
<td>6.92</td>
<td>1.92</td>
<td>9.63</td>
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<tr>
<td>$SCL_{SFC}$ (L/h)</td>
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<td>0.03</td>
<td>0.007</td>
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<tr>
<td>$V_{SFC}$ (L)</td>
<td>0.01</td>
<td>8.60 $\times$ 10$^{-3}$</td>
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<td>$K_{bcSFC}$ (h$^{-1}$)</td>
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<td>3.26</td>
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<tr>
<td>$K_{cbSFC}$ (h$^{-1}$)</td>
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<td>0.79</td>
<td>0.08</td>
</tr>
<tr>
<td>$K_{cpSFC}$ (h$^{-1}$)</td>
<td>16.84</td>
<td>17.05</td>
<td>5.29</td>
</tr>
<tr>
<td>$V_{brain}$ SFC (L)</td>
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<td>0.10</td>
<td>0.04</td>
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<td>$K_{S FC}$ llog$_{10}$CFU/g/h</td>
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<td>0.08</td>
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<tr>
<td>Interaction term SFC</td>
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<td>$-0.34$</td>
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<td>$H_{SFC}$</td>
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<td>3.44</td>
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<td>$C_{50SFC}$ (mg/L)</td>
<td>3.91</td>
<td>3.59</td>
<td>1.66</td>
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<tr>
<td>$SCL_{LAmB}$ (L/h)</td>
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<td>$0.83 \times 10^{-3}$</td>
<td>$1.81 \times 10^{-4}$</td>
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<tr>
<td>$V_{LAmB}$ (L)</td>
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<td>3.37 $\times$ 10$^{-3}$</td>
<td>0.01</td>
</tr>
<tr>
<td>$K_{bcLAmB}$ (h$^{-1}$)</td>
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<td>2.56</td>
<td>5.31</td>
</tr>
<tr>
<td>$K_{cbLAmB}$ (h$^{-1}$)</td>
<td>3.68</td>
<td>0.84</td>
<td>5.67</td>
</tr>
<tr>
<td>$K_{cpLAmB}$ (h$^{-1}$)</td>
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<td>6.38</td>
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<td>9.39</td>
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<tr>
<td>$V_{brain}$ LAmB (L)</td>
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<td>0.04</td>
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<td>$K_{L LAmB}$ llog$_{10}$CFU/g/h</td>
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<td>$H_{LAmB}$</td>
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<td>1.29</td>
<td>2.83</td>
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<td>0.05</td>
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<tr>
<td>POPMAX (CFU/g)</td>
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<td>$4.49 \times 10^{7}$</td>
<td>$3.24 \times 10^{7}$</td>
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<tr>
<td>Initial condition (CFU/g)</td>
<td>152.64</td>
<td>126.98</td>
<td>63.03</td>
</tr>
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</table>

$SCL_{LAmB}$ and $SCL_{SFC}$ are the terms for clearance for liposomal amphotericin B (LAmB) flucytosine, respectively. $V_{LAmB}$ and $V_{SFC}$ are the volume of the central compartment for LAmB flucytosine, respectively. $K_{Sfc}$, $K_{cpSFC}$, $K_{bcSFC}$, and $K_{cpLAmB}$ represent the various first-order intercompartmental rate constants. $N$ is the total number of organisms in the brain, $K_{max}$ is the maximum rate of growth of *Cryptococcus* in the brain, and POPMAX is the maximum theoretical density of organisms in the brain. $K_{SFC}$ and $K_{LAmB}$ represent the maximum rate of kill induced by concentrations of the respective agents in the brain. $C_{50SFC}$ and $C_{50LAmB}$ are the concentrations of the respective drugs in the brain at which the rate of kill is half maximal. $H$ represents the respective slope functions. L is the interaction term.

**Bridging to Humans**

The experimental murine pharmacokinetic and pharmacodynamic data were bridged to humans in order to identify potential candidate regimens for humans. The mean concentration–time profiles for humans receiving various regimens of LAmB and flucytosine were determined from respective population pharmacokinetic models published elsewhere [13, 14]. The program RightDose (www.lapk.org) was used to determine the murine dosages of each antimicrobial agent that produced human-like concentration–time profiles. RightDose is ordinarily used in clinical settings to identify human dosages required to achieve desired therapeutic plasma drug concentration levels. Once calculated, the murine dosages that produced a human-like concentration–time profile were supplied to the mathematical model (using ADAPT 5 [11]) to determine the model-predicted pharmacodynamic outcome for humans. This simulation exercise was performed with LAmB 3–6 mg/kg/d alone and in combination with flucytosine 50–100 mg/kg/d in 4 divided dosages. The endpoint was the time course of fungal infection in the brain.
untreated controls during the first 7 days of infection. The quantitative counts were reflected by histological evidence of progressive infection that manifested as multiple cystic lesions containing multiple yeasts but minimal evidence of inflammation (Figure 1). Capsular material leached into the contiguous parenchyma.

**Murine Pharmacokinetics and Pharmacodynamics of LAmB Monotherapy**

LAmB demonstrated linear pharmacokinetics (Figure 2), with rapid penetration into the cerebrum. There were quantifiable cerebral concentrations of LAmB in the first dosing interval for all dosages studied. The shape of the concentration–time
Figure 4. The pharmacodynamic data for liposomal amphotericin B and flucytosine administered alone. Data are mean ± SD. The total daily dosages of fluconazole are 75, 150, and 300 mg/kg/d.
profiles for plasma and cerebrum were similar. Estimates of the pharmacokinetic parameters for LAmB, obtained from the mathematical model, are summarized in Table 1. LAmB demonstrated dose-dependent antifungal activity (Figure 4). A dosage of 3 mg/kg/d produced submaximal antifungal effect. A dosage of 10 mg/kg/d suppressed fungal growth. The highest dosage of 20 mg/kg/d resulted in a net decline in cerebral fungal burden but did not achieve sterilization.

**Murine Pharmacokinetics and Pharmacodynamics of Flucytosine Monotherapy**

The murine pharmacokinetics of flucytosine were linear (Figure 3). Flucytosine rapidly penetrated the cerebrum, producing quantifiable concentrations in the first dosing interval for all dosages studied. The shape of concentration–time profiles in plasma and cerebrum were similar, with no evidence of hysteresis. Flucytosine did not accumulate within the cerebrum—plasma concentrations remained approximately 5 times greater than those observed in the cerebrum throughout the experimental period. Estimates of the pharmacokinetic parameters for flucytosine obtained from the mathematical model are summarized in Table 1. Flucytosine dosages of 50 and 100 mg/kg every 8 hours (ie, 150 and 300 mg/kg/d) suppressed cerebral fungal growth (Figure 4).

**Murine Pharmacokinetics and Pharmacodynamics of Combination Therapy**

There was no evidence of a pharmacokinetic interaction. Trough concentrations of both agents in combination were comparable to those found when agents were administered alone (data not shown). Using the Greco model, the combination of LAmB and flucytosine was additive, with an estimate for the interaction term that was essentially zero (Figure 5).

**Mathematical Modeling of Murine Data and Bridging to Humans**

The fit of the mathematical model to the murine data was highly acceptable. The coefficients of determination ($r^2$) for the observed-vs-predicted plots for each model output were 0.95, 0.96, 0.90, 0.60, and 0.78 before the Bayesian step for LAmB in plasma, LAmB in brain, flucytosine in plasma, flucytosine in brain, and the fungal density in the brain, respectively. There was minimal bias, and precision of the observed-vs-predicted regression was acceptable. The median parameter values of the mathematical model better accounted for the observed data and were therefore used in all subsequent analyses.

Human concentration–time profiles were constructed for LAmB alone and for LAmB combined with flucytosine. A human regimen of LAmB 3 mg/kg/d resulted in submaximal antifungal activity when compared with 6 mg/kg/d (Figure 6). The addition of flucytosine 100 mg/kg/d in 4 divided dosages (ie, 25 mg/kg every 6 hours) resulted in near maximal antifungal activity that was in excess of that observed with either dosage of LAmB used alone. Furthermore, near maximal antifungal activity was apparent with a human regimen of flucytosine 50 mg/kg/d, which is lower than currently used.

**DISCUSSION**

Lipid preparations of amphotericin B (LAmB and amphotericin B lipid complex) are increasingly used for the treatment of cryptococcal meningoencephalitis. The use of lipid formulations for cryptococcal meningoencephalitis is supported by relatively limited clinical data (eg, [4, 6]), principally because the majority of recent therapeutic studies have been performed in resource-poor settings. Our study suggests that a regimen of LAmB 3 mg/kg/d as monotherapy may be suboptimal (see Figure 6), while 6 mg/kg/d results in near-maximal antifungal effect. A recent clinical study that compared LAmB 3 mg/kg/d and 6 mg/kg/d for the treatment of cryptococcal meningoencephalitis in patients with HIV/AIDS suggests that a higher dosage is not necessarily associated with improved mycological success nor 10-week mortality [4]. However, this study was powered to detect differences in LAmB (3 mg/kg/d or 6 mg/kg/d) vs amphotericin B 0.7 mg/kg/d rather than differences between the 2 LAmB dosages. LAmB 6 mg/kg/d was associated with a
rate of renal impairment higher than 3 mg/kg/d (doubling of baseline creatinine), although this did not reach statistical significance (P = .066) [4]. LAmB also exhibits dose-dependent nephrotoxicity in other settings [16]. Therefore, use of higher...
dosages of LAmB may be possible but is limited by a higher incidence of renal impairment.

The Infectious Diseases Society of America [2] recommends the use of LAmB with or without flucytosine for patients with cryptococcal meningitis complicating HIV/AIDS, while a combination is recommended for transplant recipients. Regardless of the context, the additional value of flucytosine combined with LAmB is not well understood. Unfortunately, this is not a clinically trivial question for the following reasons: (1) flucytosine toxicity may result from renal impairment induced by any formulation of amphotericin B; (2) therapeutic drug monitoring (TDM) for flucytosine is generally required to minimize toxicity [17]; and (3) access to flucytosine is difficult in some countries. Our study strongly suggests that there is an advantage to using a combination of LAmB and flucytosine, but this is dependent on the dosage of LAmB. The combination enables attainment of maximal antifungal activity that may not be possible with LAmB 3 mg/kg/d. Furthermore, the bridging study suggests a lower dosage of 50 mg/kg/d may be reasonable and is associated with an antifungal effect that is comparable to the currently recommended regimen of 100 mg/kg/d. Consistent with this finding, a previous study of HIV-associated cryptococcal meningoencephalitis showed that oral flucytosine 100 mg/kg/d was associated with significantly lower serum and CSF concentrations compared with intravenous dosing. However, this still resulted in the same enhancement of fungicidal activity in combination with amphotericin B deoxycholate [18]. Thus, use of a lower dosage of flucytosine may be possible and may mean that TDM is only required in a subset of patients.

This study provides the experimental foundation for further clinical studies to examine the use of LAmB alone and in combination with flucytosine. Regimens associated with near maximal antifungal activity include LAmB 6 mg/kg/d alone, LAmB 3 mg/kg/d plus flucytosine 100 mg/kg/d (administered in 4 divided dosages), and LAmB 3 mg/kg/d plus flucytosine 50 mg/kg/d (administered in 4 divided dosages). The use of LAmB 3 mg/kg/d is associated with submaximal activity. A clinical study could use the rate of decline in fungal burden in the CSF as the primary endpoint, which may be a more sensitive measure of antifungal activity than microbiological clearance, clinical response, or mortality.

We acknowledge several potential limitations of our study. There is an underlying assumption that the rate and extent of trafficking of both antifungal agents from the serum into the cerebrum is comparable in mice and humans. The pharmacodynamic endpoint used in this study was the fungal density in the cerebrum, rather than the CSF. While humans with cryptococcal meningoencephalitis undoubtedly have involvement at both sites, treatment decisions for humans are based on microbiological findings from the CSF alone. The pharmacodynamic relationships for these 2 central nervous system subcompartments may differ. We investigated only a single strain of C. neoformans and therefore did not explore the potential impact of strain-to-strain variability on antifungal pharmacodynamics. Nonlinear pharmacokinetic behavior has been described in some clinical studies for patients receiving LAmB ≥7.5 mg/kg that has been attributed to saturable uptake of drug by the reticuloendothelial system [19]. Without a robust population pharmacokinetic model describing this behavior, the antifungal activity resulting from higher dosages of LAmB may be difficult to predict. The impact of the induction regimen on longer-term outcomes is not known and was not assessed in this study (ie, in terms of fungal regrowth or survival). Finally, this study did not incorporate an estimate of human pharmacokinetic variability when bridging the experimental data from mice to humans.

Despite these limitations, this study is the first to use modern pharmacokinetic–pharmacodynamic modeling and bridging techniques to identify candidate regimens for further study in clinical trials. A regimen of LAmB 3 mg/kg/d with flucytosine 50 mg/kg/d is associated with near maximal antifungal activity and likely to be significantly less toxic than other dosages of agents that are used to treat cryptococcal meningoencephalitis. This study provides the experimental foundation for further clinical studies of lipid amphotericin products and a way in which many challenges related to this neglected and frequently lethal infection can be addressed.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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