Experimental Models of Short Courses of Liposomal Amphotericin B for
Induction Therapy for Cryptococcal Meningitis

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Cryptococcal meningoencephalitis is a rapidly lethal infection in immunocompromised patients. Induction regimens are usually administered for 2-2 weeks. The shortest effective period of induction therapy with liposomal amphotericin B (LAmB) is unknown. The pharmacodynamics of LAmB were studied in murine and rabbit models of cryptococcal meningoencephalitis. The concentrations of LAmB in plasma and brain of mice were measured using HPLC. Histopathological changes were determined. The penetration of LAmB into the brain was determined by immunohistochemistry using an antibody directed to amphotericin B. A dose-dependent decline in fungal burden was observed in the brain of mice with near-maximal efficacy achieved with LAmB 10-20 mg/kg/day. The terminal elimination half-life in brain was 133 hours. The pharmacodynamics of a single dose of 20 mg/kg was the same as 20 mg/kg/day administered for 2 weeks. Changes in quantitative counts were reflected by histopathological changes in the brain. Three doses of LAmB 5 mg/kg/day in rabbits were required to achieve fungicidal activity in cerebrospinal fluid (cumulative AUC 2500 mg.h/L). Amphotericin B was visible in the intra- and perivascular spaces, leptomeninges and choroid plexus. The prolonged mean residence time of amphotericin B in the brain suggest abbreviated induction regimens of LAmB are possible for cryptococcal meningoencephalitis.

Key words. Liposomal amphotericin B, pharmacokinetics, pharmacodynamics, Cryptococcus neoformans, cryptococcal meningitis, meningoencephalitis
INTRODUCTION

Cryptococcal meningitis is a common and frequently lethal disease in patients with HIV/AIDS (1). Rapid fungicidal activity in cerebrospinal fluid (CSF) is associated with better clinical outcomes and improved survival (2). Amphotericin B deoxycholate (DAmB) is the most potent amphotericin B formulation on a mg-mg basis (3, 4). While effective, DAmB is toxic and associated with significant infusion-related toxicity, nephrotoxicity and anemia (5, 6). Furthermore, DAmB is not orally bioavailable, and must be injected. The need for rapid reliable monitoring for side effects and for intravenous administration means that amphotericin B-based treatment is simply not possible in many resource-poor settings. Hence, the best current therapy cannot be administered to patients in many countries where the prevalence of cryptococcal meningitis is the highest. In these cases, the only alternative agent is fluconazole, but even with the use of high doses (800-1200 mg/day), fungicidal activity in CSF and clinical outcomes are suboptimal (7, 8). Alternative approaches are urgently required.

There is surprisingly little evidence for the use of liposomal amphotericin B (LAmB) for cryptococcal meningitis. Preclinical and clinical data suggest 3-6 mg/kg/day is a safe and effective regimen (9, 10). Typically, the duration of amphotericin B-based induction regimens is 2 weeks, primarily based on surrogate mycological markers of early fungicidal activity such as CSF sterilization (11, 12). The shortest duration of LAmB that is maximally effective is not known. We recently demonstrated that an abbreviated course of DAmB (3 days) may be as effective as 2 weeks of therapy (13) and short courses of DAmB (in combination with fluconazole) are associated with rapid clearance of the CSF in patients with cryptococcal meningitis (14). Thus, there...
is a precedent and rationale for examining the safety and efficacy of abbreviated regimens of LAmB as induction therapy for cryptococcal meningitis.

Here, we used two previously described (15, 16) and well-characterised laboratory animal models of cryptococcal meningitis to study the pharmacodynamics of abbreviated courses of liposomal amphotericin B. Our principal goal was to provide the experimental evidence underpinning Phase II and III clinical trials examining the efficacy of abbreviated regimens of LAmB.

**METHODS**

**Strain and In vitro Susceptibility Testing**

*Cryptococcus neoformans* var. *grubii* (ATCC 208821 or H99) was the challenge organism for experiments in mice and rabbits. The minimum inhibitory concentration (MIC) testing was performed using European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical Laboratory Sciences Institute (CLSI) methodology. MICs were determined in three independently conducted experiments.

**Laboratory Animal Models of Cryptococcal meningoencephalitis.**

All murine studies were performed under UK Home Office project licence PPL 40/3630 and received prior approved by the ethics committee at the University of Liverpool. Two models of cryptococcal meningitis were used that provide complementary information on the time course of cryptococcal meningoencephalitis, and the response to treatment with LAmB. The murine model has the advantage of being highly reproducible. In this model, the fungal burden in the cerebrum is the primary read-out and quantitative counts in the CSF cannot be obtained. In contrast,
the rabbit model enables the time course of fungal burden in the CSF to be determined, which is a clinically relevant sub-compartment within the central nervous system. The fungal burden in other central nervous system sub-compartments is also available (e.g. cerebrum, vitreous, meninges), but only at the time of sacrifice.

For the murine model, immunosuppression is not required because mice are inherently susceptible to disseminated cryptococcal infection. An inoculum of $3 \times 10^8$ CFU in 0.25 mL PBS was injected i.v. via the lateral tail vein, which results in a highly reproducible encephalitis. Mortality occurs at the latter part of the second week of infection meaning that early death does not confound any assessment of fungal burden in the initial 7-10 days of infection. The intended inoculum was confirmed using quantitative counts after each experiment. The limit of detection for quantitative culture was $1.2 \log_{10}$CFU/g.

A rabbit model of cryptococcal meningoencephalitis that was originally developed and described by Perfect et al. (15) was used to study the impact of abbreviated regimens on the time course of fungal burden in the CSF. Briefly, C. neoformans inocula were grown in Yeast Extract-Peptone-Dextrose (YPD) broth to a final concentration of $3 \pm 0.25 \times 10^8$ CFU/mL. Inoculum concentrations were estimated by optical density and confirmed by quantitative culture. Anaesthetized rabbits were infected via cisternal injection with an inoculum volume of 0.3 mL. Rabbits were anesthetized at day +2, 5, 7 and 11 post-inoculation. CSF was obtained via cisternal puncture. A 1 mL sample was removed at each time point. All rabbits were sacrificed at the end of the experiment, which was 13 days post inoculation. A final CSF sample was obtained immediately after sacrifice. In addition, the fungal burden in the
cerebrum at the end of the study period was determined as an additional endpoint.

Representative samples of cerebrum were homogenized in 2 mL of Phosphate-
Buffered Saline (PBS). Homogenate and CSF were then plated to Sabouraud
Dextrose Agar (SDA) containing chloramphenicol.

Pharmacokinetic and Pharmacodynamic (PK-PD) Studies

The PK-PD relationships in mice were determined over the course of multiple
independently conducted experiments. The time-course of infection in the cerebrum
was determined using a destructive design in which groups of CD-1 mice (n=3 per
group) were sacrificed at predefined intervals between 0 and 240 hours post
inoculation. Treatment was commenced 24-hours post inoculation. Dose finding
studies were conducted using 0.5-20 mg/kg/day. Each experiment incorporated an
untreated control and at least two experimental arms (n=15 per arm). Each dosing
regimen was repeated in triplicate. Data from subjects requiring sacrifice on humane
grounds were included in analyses at the time of death.

The PK in plasma and cerebrum were determined in a separate experiment and once
the relevant dose-response relationships had been determined. The PK was
determined in infected mice. PK data were obtained at two intervals (immediately
following the initiation of therapy and then after 5 days of dosing). Groups of mice
(n=3) were sacrificed 0.5, 1, 2, 6 and 24 hours post drug administration. Plasma was
obtained by terminal cardiac puncture, placed immediately on ice, centrifuged and
stored at -80°C for analysis. The cerebrum was extracted at the time of necropsy
under sterile conditions. One hemisphere was submitted for quantitative cultures
while the other was stored for future measurement of amphotericin B concentrations.
PK-PD relationships were studied following various induction regimens, as follows. A single dose of 5 mg/kg was studied based on previous studies in invasive pulmonary aspergillosis. Groups of rabbits received a single dose, 3 doses of 5 mg/kg/day and daily therapy of 5 mg/kg/day.

**Measurement of amphotericin B concentrations**

The concentrations of amphotericin B were estimated using a previously described assay (3). The limit of detection was 0.05 mg/L. The intra and inter-day variation was <7%.

**Histopathology and staining of LAmB in the central nervous system of mice**

The brain was collected and placed into 10% neutral buffered formalin for histopathologic evaluation. Formalin fixed tissues were trimmed, cryoprotected by sucrose replacement, then embedded in OCT freezing media. Approximately 5 µm sections were prepared for staining.

A commercially available mouse monoclonal antibody directed against *Cryptococcus neoformans* was used to determine the extent of infection (MyBioSource, LLC, San Diego, CA). Yeasts stained consistently and intensely (4+) positive with the anti-C. neoformans antibody. The yeasts did not stain with the species-, isotype-, and concentration- matched negative control antibody (mouse IgG1 (Ms IgG1)) that was substituted for the Ms anti-C. neoformans reagent (data not shown).
Amphotericin B was visualised using an affinity-purified rabbit anti-amphotericin B antibody (Antibodies Inc., Davis, CA). Immunohistochemistry was performed using standard immunoperoxidase and alkaline phosphatase methodology, and validated by appropriate and reproducible positive and negative controls for staining amphotericin B as previously described (17, 18).

Mathematical Modelling

The murine PK and PD data from mice were modelled using a population methodology with the program Pmetrics (19). The mean drug concentration, cerebral concentration and fungal burden in the cerebrum from groups of 3 mice were used. All data were weighted by the observed variance from each group of mice for drug concentrations and fungal burden. The structural model took the form:

\[
\frac{dX(1)}{dt} = R(1) - \left( \frac{SCL}{V_c} + K_{cp} + K_{cb} \right) \times X(1) + K_{bc} \times X(3) + K_{pc} \times X(2)
\]

\[
\frac{dX(2)}{dt} = -K_{pc} \times X(2) + K_{cp} \times X(1)
\]

\[
\frac{dX(3)}{dt} = K_{cb} \times X(1) - K_{bc} \times X(3)
\]

\[
\frac{dN}{dt} = K_{\text{gmax}} \times \left( 1 - \left( \frac{X(3)}{V_m} \right) \right) \times \left( 1 - \frac{N}{\text{POPMAX}} \right) \times N
\]

Where X(1), X(2) and X(3) represent the amount of amphotericin B (mg) in the central compartment, peripheral compartment and cerebrum, respectively. N is the number of organisms in the cerebrum. R(1) represents the i.v. injection of liposomal amphotericin B (mg); Kcb, Kbc, Kcp and Kpc represent the first-order rate constants.
connecting the various compartments.  \( H_g \) is the slope function for the suppression of growth.  \( K_{g_{\text{max}}} \) is the maximum rate of fungal growth in the brain; \( V_m \) is the volume of the murine brain; \( C_{50g} \) is the concentration of amphotericin B in the brain at which there is half-maximal inhibition of growth, and \( P_{\text{op_{max}}} \) is the maximum theoretical density of organisms in the brain.

Equation 1 describes the movement of liposomal amphotericin B into and out of the central compartment (plasma).  Equation 2 describes the movement of liposomal amphotericin B into and out of the peripheral compartment.  Equation 3 describes the movement of drug into the brain.  Equation 4 describes the pharmacodynamics of amphotericin B.  This equation contains terms that describe the capacity-limited fungal growth in the brain, and drug induced suppression of the fungal growth.

The fit of the mathematical model to the combined PK and PD dataset from mice was assessed using the log likelihood value, measures of precision and bias and visual inspection of the observed-versus-predicted values both before and after the Bayesian step, and assessment of the linear regression of the observer-versus-predicted values both before and after the Bayesian step.  Inspection of the PK of liposomal amphotericin B in rabbits suggested that the volume of distribution contracted with time, as recently described by us in children (20).

\[
\frac{dX(1)}{dt} = R(1) - \left( \frac{SCL}{X(3)} + K_c p \right) \times X(1) + K_p c \times X(2)
\]
\[
\frac{dX(2)}{dt} = K_c p \times X(1) - K_p c \times X(2)
\]
\[
\frac{dX(3)}{dt} = -X(3) \times K + V_f i n
\]

with output equation
\[ Y(1) = \frac{X(1)}{X(3)} \]

Where \( X(1) \) and \( X(2) \) is the amount of liposomal amphotericin B in the central and peripheral compartment, respectively. SCL is the clearance of drug from the central compartment, and \( K_{cp} \) and \( K_{pc} \) are the two first order inter-compartmental rate constants connecting the central and peripheral compartments. \( X(3) \) is the volume of the central compartment that contracts with time according to equation 3. \( X(3) \) has an initial volume, \( V_{ini} \), which is estimated as an initial condition in Pmetrics. The volume contracts over time according to the first order rate constant \( K \). The final volume after prolonged drug administration is \( V_{fin} \). Equation 1 describes the rate of change of the amount of liposomal amphotericin B in the central compartment.

Equation 2 describes the rate of change of the amount of liposomal amphotericin B in the peripheral compartment.

**PK-PD Bridging Studies**

In order to place the experimental findings in a clinical context we bridged the preclinical PK-PD findings from mice and rabbits to patients using a previously described population PK model for liposomal amphotericin B (21). This model was used to estimate the average drug exposure (quantified in terms of AUC) resulting from various human doses.

**RESULTS**

**Dose-Exposure-Response Relationships in Mice**

Liposomal amphotericin B was well tolerated in mice with no observed toxicity following rapid intravenous (i.v.) injection. There was a clear dose-response relationship with doses of 0.5-20 mg/kg/day. Fungicidal activity was not observed.
(i.e. we did not observe a decline in log_{10}CFU/g following daily therapy). Rather, a fungistatic effect was seen whereby the infection at the time of drug administration 24 hours post inoculation was stabilised. Near maximal antifungal activity was observed following treatment with 10-20 mg/kg/day and with an AUC:MIC of approximately 100 (Figure 1).

A profound and durable antifungal effect was apparent following a single dose of 20 mg/kg in mice (Figure 3). There was no evidence of significant fungal regrowth after 240 hours of observation. The persistent antifungal effect may be explained by the long terminal half-life of amphotericin B in the plasma and cerebrum (circa. 113 hours; Figure 2).

**Histopathology and Immunohistochemistry in Mice**

The persistent antifungal effect evident from the log_{10}CFU/g data was mirrored by histopathological findings shown in Figure 3. In mice receiving vehicle only, cryptococcal meningoencephalitis manifested as a multifocal disease with cyst-like cavities filled with multiple encapsulated organisms approximately 6-10 µm in diameter. There was no evidence of an inflammatory component within or around the cavities.

Mouse liver (harvested from mice receiving a total cumulative liposomal amphotericin B dose of 225 mg/kg) was used as positive control tissue in all amphotericin B localization experiments. Moderate to marked staining of frequent Kupffer cells was observed in the positive control tissue. All other tissue elements were negative. There was no staining of Kupffer cells when a species-, isotype-, and
concentration-matched negative control antibody (rabbit IgG) was substituted for the rabbit anti-AMB reagent. Kupffer cells in control mouse liver that received 5% dextrose did not stain with rabbit anti-AMB reagent.

There was differential penetration of amphotericin B into the brain (Figure 5). Staining was apparent early (i.e. one-hour post dose) and in both intravascular and perivascular spaces, suggesting the drug crossed the blood-brain-barrier. Staining was especially prominent in blood vessels in the leptomeninges and choroid plexus, as well as small cerebral capillaries. Staining was both extra- and intra-cellular.

Granular extracellular staining was observed in and surrounding blood vessels. Intracellular cytoplasmic staining was observed in mononuclear/microglial cells. Positive circulating mononuclear cells (presumptive monocytes) were identified in cerebral capillaries. Additional extracellular staining was observed in the ventricular system associated with the ependymal lining suggesting entry into the cerebral spinal fluid. In contrast, staining was not observed in the normal cerebral tissue or in residual cryptococcomas after 10 days of treatment with LAmB at doses 10 or 20 mg/kg/day.

Mathematical Pharmacokinetic-Pharmacodynamic Model in Mice

The fit of the mathematical model to the combined murine PK-PD dataset was acceptable, even though fitting was difficult. The estimates for the parameters are summarized in Table 1. The principal challenge was modelling the depot-like effect of LAmB in the brains of mice where low drug concentrations were observed to have exerted an antifungal effect that lasted well beyond the time that liposomal amphotericin B concentrations were detectable.
Pharmacokinetic-Pharmacodynamic Relationships in Rabbits

The mean parameter values best accounted for the observed PK data. The parameter values were as follows: SCL 0.018 ± 0.008 L/h; Kcp 10.37 ± 0.416 h⁻¹; Kpc 26.09 ± 0.96 h⁻¹; K 0.093 ± 0.04 h⁻¹; Vini 4.717 ± 0.233 L and Vfin 0.003 ± 0.002 L. The coefficient of determination for the linear regression before and after the Bayesian step was 0.87 and 0.98, respectively and in both cases the intercept and slope approximated zero and one, respectively.

The pharmacodynamics in rabbits similarly illustrated the potential utility of abbreviated LAmB induction but differed somewhat to those observed in mice. A single dose of LAmB at 5 mg/kg appeared fungistatic only up to 264 hours, and did not provide a durable response in CSF or cerebrum ($\Delta \log_{10} CFU/g = 1.9 \pm 1.2$ and $\Delta \log_{10} CFU/g = 3.2 \pm 0.5$, respectively), despite higher estimated AUC₀⁻₂₄ compared to mice receiving a single dose of 20 mg/kg (820 ± 15 vs. 580 ± 30 mg.h/L). Three doses of LAmB at 5 mg/kg administered every 24 hours (and commencing 48 hours post inoculation) induced a prompt decline in fungal burden in the CSF and cerebrum ($\Delta \log_{10} CFU/mL = -2.8 \pm 0.8$ and $\Delta \log_{10} CFU/g = -0.1 \pm 0.4$, respectively). This regimen produced a cumulative total AUC₄₈⁻¹₂₀ of 2,499 mg.h/L. The effect of this abbreviated regimen in rabbits was comparable to that achieved with daily therapy (Figure 6).

The exposure-response relationships in the cerebrum of rabbits were similar. The fungal density ($\log_{10} CFU/g$ mean ± standard deviation) for controls, 5 mg/kg once, 5 mg/kg/day for three days and 5 mg/kg/day was 5.92 ± 0.55, 5.21 ± 1.20, 2.43 ± 1.23
and 2.47 ± 0.70, respectively. Thus, in comparison to the murine studies, > 1 day of therapy was required in rabbits to achieve fungicidal activity in the cerebrum and CSF.

Pharmacokinetic-Pharmacodynamic Targets and Bridging Studies

A human regimen of liposomal amphotericin B of 4 mg/kg/day produces an AUC$_{0-24}$ at steady state of ~190 mg.h/L. As can be seen in Figure 1 Panel F this is associated with near-maximal antifungal efficacy in mice receiving daily liposomal amphotericin B. A single dose of 20 mg/kg in mice (AUC$_{0-24}$ 550-600 mg.h/L) also produced near maximal antifungal activity. The bridging study in rabbits suggested that a single dose of 5 mg/kg (AUC 833 mg.h/L) was insufficient to achieve fungicidal activity. Rather, a total of three doses of 5 mg/kg/day (cumulative AUC 2499 mg.h/L) was required to achieve fungicidal activity in the CSF. Thus, there was a degree of discordance between the pharmacodynamic targets from mice and rabbits with the latter requiring slightly more drug exposure to achieve the same effect.

DISCUSSION

Amphotericin B is the most potent agent for induction therapy against Cryptococcus neoformans, and the combination with flucytosine results in the most rapid overall decline in fungal burden (22). This study suggests that abbreviated regimens of liposomal amphotericin B may be feasible. This is primarily a function of a favourable pharmacokinetic profile with long terminal elimination phases in both the plasma and brain (t$_{1/2}$ 133 hours).
The apparent discordance between plasma concentrations of liposomal amphotericin B and its persistent anti-cryptococcal activity in the central nervous system (CNS) of both mice and rabbits is of considerable interest, although the underlying mechanism driving this phenomenon is not entirely clear. One possibility is that there are a limited number of binding sites for amphotericin B in the central nervous system. Once occupied, amphotericin B does not readily disengage from its binding sites leading to a pharmacologically active depot of drug. A relatively short course of liposomal amphotericin B (e.g. 1-3 doses) is all that is required to fully occupy these binding sites and result in persistent antifungal activity. Further doses are simply redundant and only serve to increase the probability of toxicity. The persistent occupation of receptors results in a sustained antifungal response for many days even after plasma concentrations have declined to undetectable levels.

Exactly how liposomal amphotericin B traffics into the various clinically relevant effect sites is not clear. Drug penetrates into CNS sub-compartments that are structurally normal and with histological evidence of inflammation (e.g. the ependyma in Figure 5D). The immunohistochemistry studies suggest the transfer from blood to the CNS occurs relatively quickly (i.e. in the first 24 hours), but they do not enable estimates of the rate of transfer of drug. We did not observe high concentrations of drug within cryptococcomas where the blood-brain-barrier is likely significantly disrupted, even though amphotericin B was readily quantifiable in homogenates of cerebrum of mice. This is probably because the amphotericin B immunoassay is relatively insensitive. We did not see any evidence of drug being carried into cryptococcomas by inflammatory cells (the dump truck phenomenon) as...
is described for macrolides (23) although there was a very limited inflammatory
response in this model.

While the study provides the experimental foundation for the concept of using
abbreviated induction regimens of liposomal amphotericin B for cryptococcal
meningitis, there is some uncertainty about the best regimen(s) for humans. Taken in
isolation, the rabbit studies suggest that more than a single dose is required (with a
cumulative AUC >833 mg.h/L). The AUC associated with a dose of 5 mg/kg i.v. in a
rabbit is higher than that observed following 20 mg/kg i.v. to a mouse (833 versus 555
mg.h/L, respectively) for which prolonged antifungal activity in the cerebrum was
observed (Figure 1). Thus, the mouse may underestimate the total (cumulative) drug
exposure required for fungicidal activity in humans. Estimates of appropriate
regimens are further complicated by some uncertainty in the PK of higher doses of
liposomal amphotericin B in humans. We recently described much greater drug
exposures (Cmax and AUC) after multiple doses in at least some children receiving
up to 10 mg/kg of LAmB (20) although we did not observe this phenomenon with
high-dose intermittent dosing in adults (21). Further detailed PK studies of higher
doses of LAmB are warranted.

The current study has several limitations and assumptions. Firstly, we did not
examine whether immunological effectors may have had an additional antifungal
effect to that of LAmB, and whether this may have contributed to persistent
antifungal activity observed with single doses. There was no evidence from the
histopathological studies of an inflammatory infiltrate in either mice or rabbits (the
latter is not shown). We extensively investigated this possibility in a recent study that
examined the effect of abbreviated regimens of DAmB for cryptococcal
meningoencephalitis, in which there was no evidence of immune-mediated antifungal
killing (13). Secondly, we made an explicit assumption that the trafficking (both the
rate and extent) of drug from the bloodstream to the site of infection is the equivalent
in mice, rabbits and humans. Such an assumption is central to PK-PD bridging
studies for all drug-pathogen combinations. In the majority of cases, this assumption
is reasonable, but there are isolated examples where it is not (24). Thirdly, there
remains a degree of uncertainty regarding the lowest dose and shortest possible course
of LAmB that is likely to be effective for patients with cryptococcal
meningoencephalitis. We did not design this study to specifically address this
question, which would have required many more animals. Finally, we did not
examine optimal combinations of antifungal agents when one or both agents is
administered as a short course.

Given the overwhelming cost and feasibility advantages of abbreviated induction
therapy based on one or few doses of LAmB, clinical trials are now required to
further test these ideas. A two-stage adaptive open-label phase II/III randomised non-
inferiority trial comparing alternative short course LAmB regimens is underway and
will report in 2017 (trial registration number: ISRCTN10248064). These clinical trials
will provide information for new therapeutic options for this neglected infection.

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Table 1. The parameters from the population pharmacokinetic-pharmacodynamic model from mice, along with the estimates for the mean, median and standard deviation. Parameter values are as described in the text.

| Parameter (Units) | Mean   | Median  | Standard Deviation |
|-------------------|--------|---------|--------------------|
| SCL (liters/h)    | 0.00082| 0.00094 | 0.00018            |
| Volume (liters)   | 0.003  | 0.0027  | 0.0019             |
| Kcp (h⁻¹)         | 11.99  | 10.52   | 9.94               |
| Kpe (h⁻¹)         | 15.70  | 23.33   | 11.41              |
| Kcb (h⁻¹)         | 0.16   | 0.22    | 0.013              |
| Kbc (h⁻¹)         | 0.034  | 0.01    | 0.038              |
| Kgmax (log₁₀CFU/g/h) | 0.096 | 0.084   | 0.033              |
| Hg                | 7.96   | 7.72    | 6.00               |
| C₅₀g (mg/L)       | 0.088  | 0.056   | 0.100              |
| POPMAX (CFU/g)    | 23785100 | 57435030 | 249007             |
| Vm (liters)       | 0.72   | 0.94    | 0.37               |
| Initial Condition (CFU/g) | 186 | 207 | 133.97 |
Figure 1. Pharmacodynamics of liposomal amphotericin B (LAmB) in cohorts of mice receiving 0.5 (Panel B), 3 (Panel C), 10 (Panel D) and 20 mg/kg/day i.v. (Panel E). The area under the concentration time curve (AUC:MIC) at steady state versus the observed fungal density at the end of the experiment (time = 240 hours) is shown in Panel F. All data are mean ± standard deviation from groups of three mice.
Figure 2. The pharmacokinetics of liposomal amphotericin B (LAmB) in murine plasma (red line and red data points) and cerebrum (black line and black data points) in cohorts of mice infected with Cryptococcus neoformans receiving LAmB 20 mg/kg once i.v. (24 hours post inoculation). All data are mean ± standard deviation from groups of three mice. The terminal half-life in the plasma and cerebrum is circa 133 hours.
Figure 3. The pharmacodynamics of vehicle control (Panel A) and liposomal amphotericin B (Panel B) following the administration of a single dose of 20 mg/kg i.v. to mice with cryptococcal meningoencephalitis. Data are the mean ± standard deviation from groups of three mice. The solid line is the fit of the mathematical PK-PD model (parameters summarised in Table 1). The black arrow denotes the time of drug administration relative to inoculation (which occurred at time=0).
Figure 4. Representative cross sections of brains from mice receiving vehicle control, LAmB 20 mg/kg/day i.v. and LAmB 20 mg/kg once i.v. Each section has been stained with an anti-cryptococcal antibody and then counter stained with hematoxylin. Treatment was initiated at the end of Day 1 (i.e. 24 hours post inoculation). There are multiple cryptococcomas at the end of Day 7 in untreated controls. In contrast there are relatively few and exceedingly small lesions in both treatment groups.
Figure 5. Distribution of liposomal amphotericin B (LAmB) in the central nervous system. All mice received LAmB 20 mg/kg i.v. In each panel the black arrows show areas of staining of amphotericin B. Panel A, LAmB staining in the choroid plexus (extracellular and within a macrophage); Panel B, positive LAmB staining within a mononuclear cell in a thin-walled cerebral capillary; Panel C, positive staining in a perivascular location, adjacent to a thick-walled small arteriole; Panel D, LAmB staining in CSF associated with apical surface of in ependymal cells, cerebral aqueduct. Scale bars 5µm in Panels A, B and D. Scale bar 25 µm in Panel C.
Figure 6. The time course of fungal density in the CSF of rabbits following various regimens of liposomal amphotericin B. Each line represents the data from a single rabbit. Each animal received 5 mg/kg every 24 hours. The time course of fungal density in the CSF in rabbits receiving a single dose of drug (Panel B) is comparable to controls shown in Panel A. An abbreviated regimen of 5 mg/kg/day for 3 doses results in prompt fungicidal activity that is comparable to daily therapy with the same dose.
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A

Control

B

Fungal Burden (Log$_{10}$CFU/g)

Time (hours)

240

192

144

96

48

0

Fungal Burden (Log$_{10}$CFU/g)

Time (hours)

240

192

144

96

48

0

20 mg/kg once
