Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial

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Summary

Background It frequently takes more than 2 weeks for drug treatments for cryptococcal meningitis to sterilise cerebrospinal fluid (CSF). In-vitro and animal studies lend support to the use of combinations of amphotericin B, flucytosine, and fluconazole for treatment of cryptococcosis. We compared the fungicidal activity of combinations of these drugs for initial treatment of patients with cryptococcal meningitis.

Methods 64 patients with a first episode of HIV-associated cryptococcal meningitis were randomised to initial treatment with: amphotericin B (0·7 mg/kg daily); amphotericin B plus flucytosine (100 mg/kg daily); amphotericin B plus fluconazole (400 mg daily); or triple therapy with amphotericin B, flucytosine, and fluconazole. Our primary endpoint was fungicidal activity, measured by the rate of reduction in CSF cryptococcal colony-forming units (CFU) from serial quantitative CSF cultures on days 3, 7, and 14 of treatment.

Findings Baseline CSF CFU counts were an important prognostic factor. Clearance of cryptococci from the CSF was exponential and was significantly faster with amphotericin B plus flucytosine than with amphotericin B alone (p=0·0006), amphotericin B plus fluconazole (p=0·02), or triple therapy (p=0·02).

Interpretation At these doses, amphotericin B plus flucytosine is the most rapidly fungicidal regimen. Quantification of CSF cultures provides a powerful new means to accurately assess the fungicidal activity of new treatment regimens for cryptococcal meningitis.

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Introduction

Cryptococcal meningitis is a common and often fatal opportunistic infection in HIV-infected individuals, especially in Africa and Asia. In northeast Thailand, cryptococcal disease is second only to tuberculosis as an AIDS-defining illness.[1] In a Bangkok hospital, mortality of patients with HIV-associated cryptococcal meningitis was 43% with a mean time to death of 14 days, despite treatment with full conventional doses of amphotericin B.[2]

At least two factors might explain this high acute mortality: raised intracranial pressure and only moderately effective antifungal regimens, which frequently take more than 2 weeks to sterilise CSF. In a multicentre trial,[3] the proportion of patients with negative CSF culture at 2 weeks was only 51% for amphotericin B and 60% for amphotericin B plus flucytosine (difference borderline p=0·06). In a separate study,[4] 2 week CSF culture status was an important determinant of outcome at 10 weeks by multivariate analysis. In-vitro and animal studies of the treatment of cryptococcosis have lent support to the use of antifungal combinations, including amphotericin B plus fluconazole.[5,6] Fluconazole is now widely available and affordable. Therefore, we examined whether initial drug combinations that include fluconazole lead to more rapid CSF sterilisation without additional toxicity. We measured CSF sterilisation rates using a new primary endpoint, the rate of reduction in CSF colony forming units (CFU) defined by serial quantitative CSF cultures.

Materials and methods

Participants and procedures

The study was done at Sappasitprasong Hospital, Ubon Ratchathani, northeast Thailand, and approved by the ethical and scientific review subcommittee of the Thai Ministry of Public Health and by the research ethics committee of St George's Hospital, London, UK. Between May and December, 2002, after obtaining written informed consent, we enrolled 64 adults with a first episode of cryptococcal meningitis, diagnosed by CSF India ink and cryptococcal antigen tests (figure 1). Exclusion criteria were alanine aminotransferase concentration more than five times the upper limit of normal, neutrophil count less than 500×10^6/L, platelet count less than $50\,000\times10^6$/L, pregnancy, or previous serious reaction to study drugs. The participants were randomised in blocks of 16 by means of numbers in sealed envelopes prepared by an independent person to give equal numbers in each of four treatment arms: amphotericin B deoxycholate (0·7 mg/kg daily, Fungizone, Bristol-Myers Squibb, New York, NY, USA) alone; amphotericin B plus flucytosine (100 mg/kg daily, Ancotil, International and Chemical Nuclear, now called Valeant, Basingstoke, UK); amphotericin B plus fluconazole (400 mg daily, Diflucan, Pfizer, New York, NY, USA); and triple therapy with amphotericin B, flucytosine, and fluconazole. Treatment was not blinded. Unless contraindicated, patients received 1 L of 0·9% (normal)

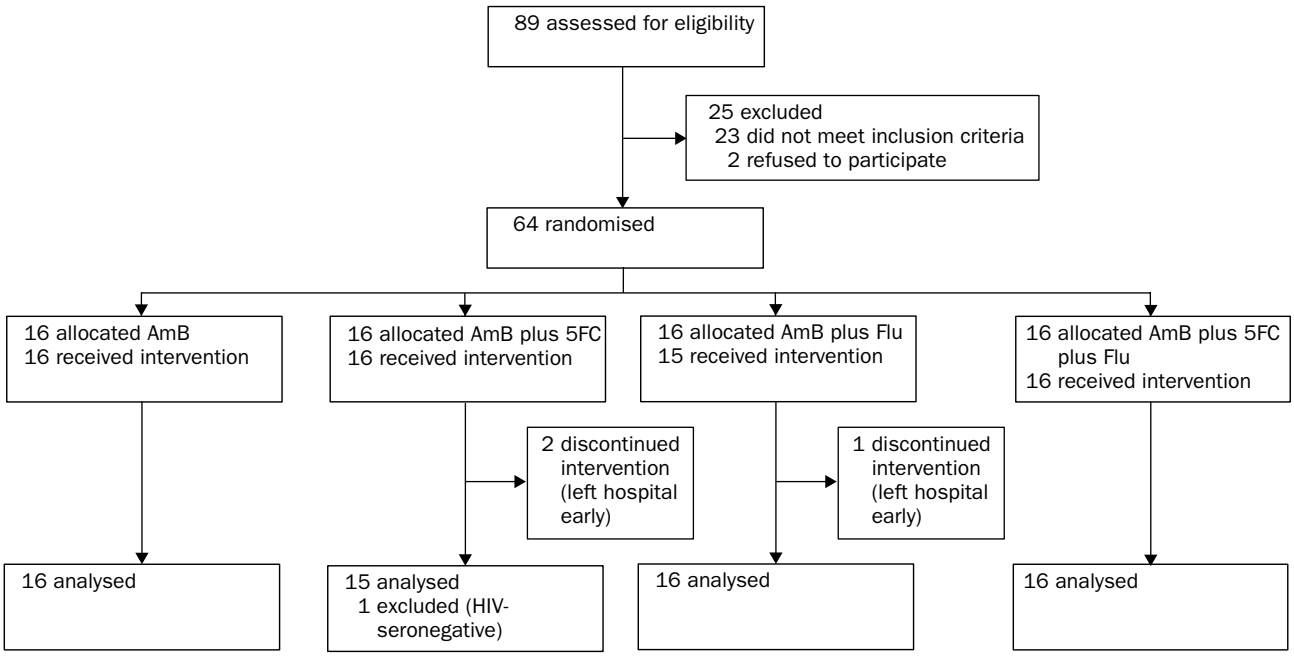

Figure 1: **Trial profile**
AmB=amphotericin B. 5FC=flucytosine. Flu=fluconazole.

saline daily to keep amphotericin B nephrotoxicity to a minimum. After 2 weeks, we treated all four arms with fluconazole, 400 mg daily for 8 weeks, and 200 mg daily thereafter (Fluzoral, Government Pharmaceutical Organisation, Thailand).

We did follow-up lumbar punctures on days 3, 7, and 14. Patients with high CSF opening pressure had additional lumbar punctures in accordance with current guidelines. With a mean delay of less than 2 h after lumbar puncture, CSF was serially diluted tenfold, and 100 μL of each dilution spotted onto each half of a Sabouraud's dextrose agar plate. We took counts from the plate with the lowest dilution that had at least 40 colonies. We monitored patients in hospital for 2 weeks. Outpatient follow-up was in an established HIV clinic and was complete to 10 weeks. At the time of the study, antiretroviral treatment was not generally available in Thailand, so no patients received antiretroviral treatment within 10 weeks of the diagnosis of cryptococcal meningitis.

Statistical analysis

We compared baseline characteristics of groups by use of the χ^2 test or Fisher's exact test for categorical variables, and the Kruskal-Wallis test or ANOVA for continuous variables. Clinical outcomes were compared between groups by Fisher's exact test. We used Fisher's exact test, the Mann-Whitney *U* test, and logistic regression to determine factors associated with death by 10 weeks. A linear regression model was used to compare the rates of decline in CSF CFU between treatment groups.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or the writing of the report.

	All patients	AmB	AmB plus 5FC	AmB plus Flu	AmB plus 5FC/Flu	p
Male (%)	38 (60%)	8 (50%)	8 (53%)	12 (75%)	10 (63%)	0·55
Age (years)‡	33 (29–36)	34 (28–38)	34 (29–36)	34 (29–38)	32 (30–34)	0·93
Mean (SD) weight (kg)	47 (8·6)	46 (8·3)	48 (8·8)	46 (6·8)	49 (10·3)	0·56
Known HIV-seropositive at presentation	40 (63%)	10 (63%)	10 (67%)	11 (69%)	9 (56%)	0·89
Previous AIDS diagnosis*	28 (44%)	8 (50%)	5 (33%)	8 (50%)	7 (44%)	0·76
Any decrease in Glasgow Coma Scale, or seizures, at presentation	12 (19%)	1 (6%)	2 (13%)	6 (38%)	3 (19%)	0·17
CD4 count (×10⁹/L)‡	9 (6–20) (n=41)	9 (6–20) (n=10)	12 (8–17) (n=9)	13 (6–23) (n=10)	10 (7–32) (n=12)	0·95
CSF						
OP (cm)‡	25 (17–32) (n=56)	21 (19–30) (n=14)	20 (14–27) (n=13)	29 (20–33) (n=15)	28 (24–36) (n=14)	0·28
White cell count/mL‡	3 (0–100) (n=60)	1 (0–230) (n=16)	4 (0–100) (n=14)	9 (0–54) (n=14)	5 (0–150) (n=16)	0·97
Crypto Ag titre‡	1024 (256–2048) (n=61)	512 (256–1024) (n=16)	384 (128–1024) (n=14)	1024 (512–2048) (n=16)	1024 (512–4096) (n=15)	0·12
QC (CFU/mL CSF)‡	537 500 (53 500–1 995 000)	422 500 (154 500–930 000)	357 500 (5500–910 000)	710 000 (22 100–3 275 000)	1 370 000 (172 000–2 150 000)	0·62
(n, n>5×106)	(62, 3)	(16, 1)	(13, 0)	(15, 2)	(16, 0)	
Deaths						
2 weeks	9	2	1	5	1	0·28
10 weeks†	14	3	1	7	3	0·11

Data are number or number (%), unless otherwise stated. AmB=amphotericin B. 5FC=flucytosine. Flu=fluconazole. OP=opening pressure. Crypto Ag=cryptococcal antigen. QC=quantitative culture. Follow-up to 10 weeks was complete. *Wasting syndrome 11 (17%), tuberculosis nine (14%), Pneumocystis carinii pneumonia six (10%), cerebral toxoplasmosis one (2%), penicilliosis one (2%). †Includes deaths within 2 weeks. ‡Median (IQR).

Baseline clinical and laboratory characteristics and clinical outcomes

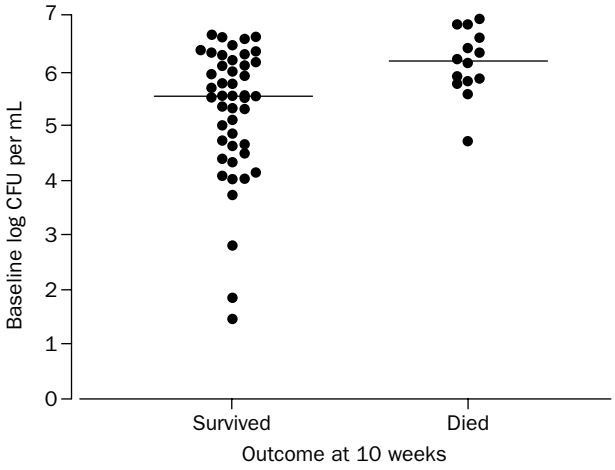

Figure 2: **Baseline quantitative CSF culture and death at 10 weeks**
Horizontal lines indicate median values.

Results

The table shows baseline clinical and laboratory characteristics and clinical outcomes. At time of presentation with cryptococcal meningitis, 40 (63%) patients were known to be HIV-seropositive and 28 (44%) had a previous diagnosis of AIDS. The most common previous AIDS-defining illnesses were wasting syndrome, tuberculosis, and *Pneumocystis carinii* pneumonia. The median CD4 count was 9×10^9/L. All treatments were well tolerated and no drug treatment had to be withdrawn within the first 2 weeks because of side-effects. There was no severe bone-marrow depression (platelets $<50\,000\times10^6$/L, neutrophils $<500\times10^6$/L) with flucytosine and no clinically significant rises in liver function tests (>fivefold above normal). Mortality was 14% (nine/63) at 2 weeks and 22% (14/63) at 10 weeks, which compares favourably with previous series from Thailand, but relates to the late presentation of this infection and the fact that severely ill patients were included.

Two factors were associated with increased risk of death by 10 weeks under univariate analysis: cerebral dysfunction defined beforehand as seizures or diminished level of consciousness at presentation (six of 49 survivors *vs* six of 14 who died, p=0·018, Fisher's Exact test), and baseline log CFU per mL (p=0·003, Mann-Whitney *U* test, figure 2). In a logistic regression model including both these factors, cerebral dysfunction and log cryptococcal CSF CFU per mL remained independently associated with early death, with odds ratios of 9 (95% CI 1·5–55·0, p=0·015) and 4 (1·4–11·0, p=0·01), respectively. Adjustment for age, sex, and other variables had no substantial effect on these estimates. All six patients with cerebral dysfunction and a CSF CFU count above the median died within 1 week. The three patients with CSF CFU above 5×10^6/mL died within 3 days. Treatment group was not significantly associated with

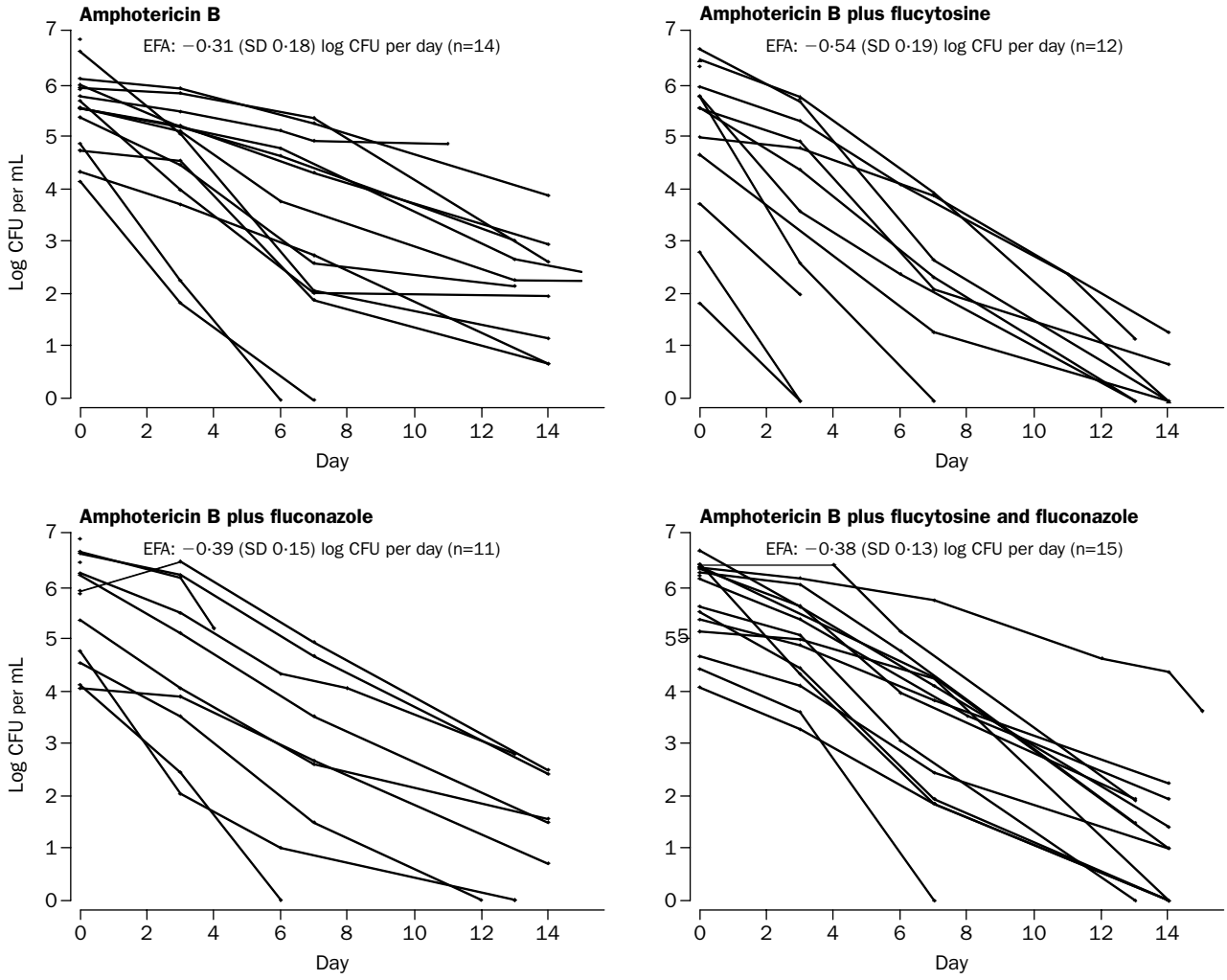

Figure 3: **Fall in CSF CFU over time by treatment group**

death at 10 weeks. There were more deaths in the amphotericin B plus fluconazole group, but this group also had more patients with severe disease (table).

The reduction in CSF cryptococcal CFU was exponential, consistent with our previous observations.[7] We calculated a summary statistic for each patient, the initial rate of reduction in CSF CFU, defined as the decrease in log CFU per mL CSF per day derived from the slope of the linear regression of log CFU against time. Negative CSF cultures were assigned a value of 1 CFU per mL. We used all data points except for sterile cultures at 14 days if this value reduced the slope. In these cases CSF sterility would probably have been achieved some time between days 7 and 14, and use of the day 14 result would therefore underestimate the true slope.

The mean rate of fall in CSF log CFU counts, or early fungicidal activity, ranged from over half a log reduction in CSF CFU per day for amphotericin B plus flucytosine to less than a third of a log reduction in CSF CFU per day for amphotericin B alone (figure 3). In a linear regression model early fungicidal activity was strongly associated with treatment group (p=0·007). Amphotericin plus flucytosine had significantly higher early fungicidal activity than did amphotericin alone (difference=0·23 log CFU daily [95% CI 0·10–0·36], p=0·001), amphotericin B plus fluconazole (difference=0·15, [0·01–0·29], p=0·03), or triple therapy with amphotericin B, flucytosine, and fluconazole (difference=0·17, [0·04–0·30], p=0·01) (figure 3).

There was a weak association between rate of fall in CFU and baseline log CFU, classified into quartile groups, such that patients with high baseline counts tended to have slower falls in CFU (reduction in rate of fall for each increment in baseline log CFU quartile=0·05 log CFU daily [0·001–0·09], p=0·04). The rate of fall was not associated with other patient characteristics. Inclusion of log baseline count in the linear regression model did not substantially alter the differences between the drug regimens: early fungicidal activity for amphotericin B plus flucytosine remained significantly greater than with amphotericin B alone (difference=0·23 log CFU daily [0·10–0·35], p=0·0006), amphotericin B plus fluconazole (difference=0·15 [0·02–0·29], p=0·02), and triple therapy with amphotericin B, flucytosine, and fluconazole (difference=0·16 [0·04–0·28], p=0·02).

Discussion

Our study confirms the greater fungicidal activity of amphotericin plus flucytosine compared with amphotericin B alone in cryptococcal meningitis, as suggested by the Mycoses Study Group trial,[3] and shows that flucytosine can be used safely in the setting of a provincial hospital in Thailand. Concern over combining amphotericin B and fluconazole has been based on their related effects on fungal membrane ergosterol. Our results are consistent with previous in-vitro and animal data, suggesting some additive effect for this combination against *Cryptococcus neoformans*.[5,6] By contrast, at the doses given, triple therapy was only as effective as amphotericin B plus fluconazole, and was significantly less fungicidal than amphotericin B plus flucytosine. This is consistent with animal model data,[6] suggesting lack of additional activity when the three drugs are combined compared with the two drug combinations, and with in-vitro studies that failed to show consistent additive or synergistic interaction between flucytosine and fluconazole against *C neoformans*.[8]

Previous therapeutic studies in cryptococcal meningitis have shown whether cultures are positive or negative at necessarily small numbers of time points. Serial quantification of viable CSF cryptococci allows accurate assessment and comparison of the fungicidal activity of different treatment regimens in small numbers of patients, and could aid priority setting for which new drugs or combinations should move forward to much larger clinical endpoint trials. Further work is needed to characterise the relation between cryptococcal clearance rates and clinical outcomes, and to define drug regimens with greater fungicidal activity than amphotericin B plus flucytosine.

Contributors

All authors contributed to the design of the study. A Brouwer, A Rajanuwong, W Chierakul, and T Harrison did the study. A Brouwer and T Harrison analysed the data. A Brouwer, N White, and T Harrison wrote the report.

Conflict of interest statement

None declared.

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