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Short course amphotericin B with high dose fluconazole for HIV-associated cryptococcal meningitis

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Summary *Objective:* To define more rapidly effective initial antifungal regimens sustainable in resource-constrained settings.
Methods: Cohort study in SW Uganda: Thirty HIV-seropositive, antiretroviral therapy-naïve, patients with first episode cryptococcal meningitis were treated with high dose fluconazole (1200 mg/d for 2 weeks, then 800 mg/d until ART started) plus amphotericin B (AmB, 1 mg/kg/d), with routine normal saline and potassium supplementation, for the initial 5 days. Outcome measures were early fungicidal activity (EFA), determined by serial quantitative CSF cultures, safety, and mortality.
Results: EFA was -0.30 ± 0.11 log CFU/day calculated over the first 2 weeks of treatment, with no reduction in the rate of clearance between days 5 and 14. There was no grade IV hypokalemia or elevated creatinine, and no grade III or IV anemia or elevation of ALT. AmB or high dose fluconazole were not stopped early in any patient. Mortality was 23% at 2, and 28% at 10 weeks.
Conclusions: Short course AmB was associated with rapid clearance of infection and was well-tolerated, suggesting it could be used safely in many centres currently relying on fluconazole monotherapy. Phase III trials are needed in African centres to compare short course with the standard 2-week course of AmB.

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Introduction

Cryptococcosis remains a common opportunistic infection and a common cause of death in HIV-infected patients.[1,2] Despite expansion of ART programmes, cases have not decreased in most African centres.[3] The high mortality associated with cryptococcal meningitis, recently estimated at up to 500,000 deaths per year in Sub-Saharan Africa,[2] is driven by the high case fatality rate, which, in turn, is at least partly due to the lack of effective antifungal regimens suitable for resource-constrained settings.

A 2-week initial induction with intravenous amphotericin B (AmB) is widely accepted as the gold standard therapy for HIV-associated cryptococcal meningitis if resources and facilities allow.[4,5] Unfortunately in many centres in Africa, 2 weeks induction therapy with AmB may be difficult to sustain. In addition to the costs of the drug, which may be substantial in local terms,[6] are the requirements for hospitalization, intravenous drug administration, including nursing time and expertise in positioning and maintaining intravenous access for a drug which causes considerable phlebitis, saline fluids, and electrolyte replacement, and regular, rapid and reliable laboratory monitoring for renal function, electrolytes, and hemoglobin.

As a consequence, many centres have continued to rely on fluconazole, available free through a donation program, as initial treatment. But 10 week mortality with fluconazole, at up to 800 mg/d, is more than 50%.[7–9] In addition to directly contributing to poor clinical outcome, the inadequate antifungal activity of fluconazole monotherapy may predispose to the development of secondary fluconazole resistance,[10] and potentially could also increase the risk of immune reconstitution reactions following introduction of ART.

We have shown, using serial quantitative cerebrospinal fluid cultures, that fluconazole at 400 mg/d is only fungistatic.[8] And, in a dose escalation cohort study in Mbarara Uganda, even with doses up to 1200 mg/d, the rate of clearance of infection is less rapid than AmB-based treatment.[9] Supporting the clinical relevance of such rate of clearance of infection studies, we demonstrated that this rate is independently associated with 2 and 10-week mortality in a combined cohort that now totals over 300 patients.[11,12] Therefore, we have explored strategies for further enhancing the antifungal activity of high dose fluconazole (1200 mg/d), that also remain potentially sustainable in resource-limited centres: Addition of flucytosine to fluconazole in an optimised oral regimen was shown to markedly enhance rate of clearance of infection and to be associated with a strong trend towards improved 2 week survival.[12] Herein, we report on a new cohort of patients treated in Mbarara, Uganda with an alternative strategy: addition of a short course (5 days) of AmB to high dose fluconazole.

Evidence suggests AmB and fluconazole (800 mg/d) is more effective than AmB alone.[13] In addition, a short course of AmB would be much more easily implemented than a full, standard 2-week course in resource-limited settings, and may not be associated with a significant reduction in efficacy, given the long half life of AmB and if induction therapy is continued with optimized oral therapy. A very significant reduction in the burden of infection in terms of the CSF colony-forming units (CFU) counts of between 3 and 4 logs can be achieved with 7 days of AmB at 1 mg/kg/d.[8] In addition, renal impairment and anaemia, which are dose-related, and usually manifest during the second week of induction,[14] may be reduced, and iv access is much easier to maintain, and line infections may be reduced, with short course rather than 14 days AmB treatment.

In this study AmB was closely monitored. However, in addition to determining rate of clearance of infection and clinical outcomes, an important objective of the study was to assess the tolerability of short course AmB, and how often early discontinuation, and adjustments in potassium supplementation were needed, so as to assess whether, if in wider implementation monitoring was not to a study standard, short course AmB could still be introduced and given safely.

Patients and methods

Participants

The study was conducted at Mbarara University Hospital, Mbarara, in Southwest Uganda, and approved by the research ethics committees of Mbarara University of Science and Technology and Wandsworth LREC (covering St Georges University of London, UK), and by the Uganda National Council for Science and Technology.

HIV-infected patients aged $\geq$18 hospitalized with a first episode of CM diagnosed by CSF India ink were eligible for enrolment. The diagnosis of CM was confirmed by positive CSF culture for *C. neoformans*. Patients were excluded if they had ALT $>$5 times upper limit of normal ($>$200 IU/L), were pregnant, had a previous serious reaction to fluconazole, had been on fluconazole in the last 1 month, had creatinine $>$ 2.5 mg/dl, or were already on antiretroviral therapy (ART). These criteria, apart from addition of the creatinine criterion, were the same as in earlier historical cohorts enrolled at the same centre and treated with high dose fluconazole.[9] Written informed consent was obtained from each patient, or next of kin for patients with altered mental status.

Intervention

Patients were treated with oral fluconazole (Diflucan, Pfizer, New York, USA) 1200 mg/day for the first 2 weeks in combination with amphotericin B 1 mg/kg/day (IV, Fungizone, Bristol Myers Squibb) for the first 5 days. During the first 5 days, 1 L of normal saline plus 20 mmol potassium chloride was given daily, unless contraindication, and in addition to standard fluid replacement in those patients with inadequate fluid intake, prior to AmB administration, to minimise AmB nephrotoxicity and help maintain serum potassium. Follow-up lumbar punctures were performed on days 3, 7 and 14. Patients with high CSF opening pressure and/or headache or other symptoms attributable to raised pressure had additional lumbar punctures.[4]

After 2 weeks, fluconazole was reduced to 800 mg/d until initiation of ART (nevirapine or efavirenz, plus stavudine and lamivudine according Ugandan Ministry of Health guidelines, at a median (IQR) of 33 (28, 37) days

after the start of antifungal therapy), then 400 mg/d to complete 10 weeks of antifungal therapy, and 200 mg/d thereafter. Fluconazole dosage was adjusted in patients with significant renal impairment, and consolidation and maintenance dosage (after the first 2 weeks) increased 50% in those on concomitant rifampicin.[12] Participants were discharged when clinically stable after completion of AmB therapy, and followed up for 10 weeks from enrolment.

Evaluation and outcomes

All participants had baseline full blood count, electrolytes, creatinine, ALT, and CD4 cell count. Electrolytes and creatinine were repeated on days 3, 5, 7 and 14, and full blood count and ALT on days 7 and 14.

CSF samples were analysed for cell count and differential, protein, glucose, India ink, and quantitative fungal culture, as previously described.[15] Cryptococcal clearance rates were calculated using a summary statistic for each patient, defined as the decrease in log CFU per ml CSF per day using the slope of the linear regression of log CFU against time for each patient.[15]

The mean rate of decrease in CSF cryptococcal CFU, or early fungicidal activity (EFA), was compared to prior historical cohorts of patients treated at the same centre with fluconazole alone at 800 and 1200 mg/d.[9] Laboratory toxicities were graded according modified Division of AIDS criteria, and percent changes in laboratory parameters calculated using the following formula: (final value-baseline value/baseline value) x 100%. The frequency with which early discontinuation of AmB or adjustment in potassium supplementation was required was recorded, as well as mortality at 2 and 10 weeks.

Statistics

Linear regression was used to compare the EFA in this cohort with the 2 prior historical cohorts of patients treated with fluconazole at the same centre,[9] adjusting for other variables where indicated, giving summary differences with 95% CI and significance levels.[15] To aid in the assessment of the validity of this historical comparison, baseline characteristics in this cohort were compared with the prior cohorts using the Chi-square test for categorical variables, and the Mann Whitney *U* test for continuous variables. Analyses were performed using Stata version 11 (Stata statistical software, Stata Corp., College Station, Texas, USA).

Results

Between February 2008 and November 2009, 64 patients with a positive CSF India Ink result were screened. 34 patients were excluded, in all cases because they were already on ART, and 30 patients were enrolled in the study. One patient was lost to follow up between 2 and 10 weeks of treatment. Apart from median CD4 cell count, which was higher in this cohort, there were no significant differences in baseline variables compared with the prior 2 cohorts of patients treated with fluconazole at the same centre[9] (Table 1). The median (IQR) length of admission was 9 (7.5, 13) days.

Early fungicidal activity

The mean rate of decrease in CFU per ml CSF per day, or early fungicidal activity (EFA), was -0.30 ± 0.11 log CFU/day calculated over the first 2 weeks of treatment, and -0.31 ± 0.14 log CFU/day calculated over the first week (Fig. 1). This rate of clearance of infection over 2 weeks was significantly more rapid than in the prior cohorts of patients treated at the same centre with high dose fluconazole alone: vs cohort treated with fluconazole 800 mg/d, difference 0.23 log CFU/day, 95%CI 0.15—0.31; vs cohort treated with fluconazole 1200 mg/d, difference 0.12 log CFU/day, 95%CI 0.06—0.17; $p < 0.001$ for both comparisons. Adjusting for CD4 cell count made no difference to these estimates (vs cohort treated with fluconazole 800 mg/d, difference 0.22 log CFU/day, 95%CI 0.14—0.30; vs cohort treated with fluconazole 1200 mg/d, difference 0.11 log CFU/day, 95%CI 0.06—0.16; $p < 0.001$ for both comparisons).

Table 1 Baseline clinical and laboratory characteristics for this cohort compared with the prior cohorts of patients treated at the same centre with high dose fluconazole alone.[9] N(%) for categorical variables, medians (interquartile range) for continuous variables. OP = opening pressure.

	Short course AmB	Fluconazole (800, 1200)[a]	*P*
n	30	60	
Male (%)	21 (70%)	34 (57%)	0.26
Age (yrs)	35 (33—40)	34 (29—39)	0.23
Weight (kg)	50 (45—60)	48 (44—55)	0.23
Abnormal mental status[b] (%)	17 (57%)	28 (47%)	0.37
CD4 count ($\times 10^6$/L)	21 (12—62)	12 (4—32)	0.01
CSF data			
OP (cm H_20)	28 (22—36)	30 (22—40)	0.31
White cell count	10 (10—15)	15 (7—30)	0.57
Baseline fungal burden (log CFU/ml CSF)	5.2 (4.5—6.3)	5.8 (5.1—6.2)	0.26

[a] Data from.[9]
[b] Defined as any reduction in Glasgow Coma Score.

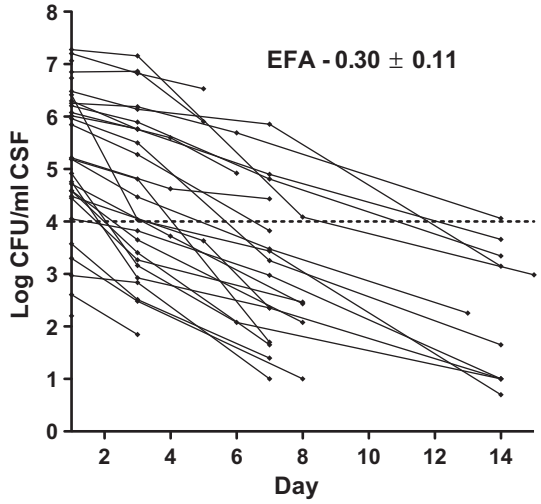

Figure 1 Fall in CSF *C. neoformans* CFU over time. The decrease in log CFU per ml CSF per day was calculated for each patient using the slope of the linear regression of log CFU against time. Early fungicidal activity (EFA) is shown as the mean rate of fall in log CFU counts/day.

Mortality

The overall mortality was 23% (7/30) at 2 weeks and 28% (8/29, 1 lost to follow up) at 10 weeks. 7 of the 8 deaths were thought related to cryptococcal infection.

Safety

None of the patients developed any serious adverse event necessitating alteration or termination of either amphotericin B or high dose fluconazole.

With just 5 days AmB and regular addition of 20 mmol potassium in the extra 1 L of normal saline given per day, serum potassium was well-maintained. Median (IQR) serum potassium at baseline was 3.8 (3.2—4.2) mmol/L and at day 5 3.7 (3.5—4.2) mmol/L. Only 2 patients developed grade III hypokalaemia, and one of these was on day 14 and may not have been AmB-related. In no patient was the i/v potassium held because of hyperkalemia, and in only one case was extra oral potassium given. Median (IQR) creatinine was 0.8 (0.6—1.1) mg/dl at baseline, 1.1 (1.0—1.4) mg/dl at day 7, and 1.0 (0.8—1.4) mg/dl at day 14. Only 2 patients developed a creatinine level greater than twice the baseline level in the first 2 weeks of treatment (Table 2). There was no grade III or IV anemia, or elevation of ALT. Hemoglobin levels dropped by 8% at day 7 and 6% at day 14 (Table 2). Median ALT was 33, 27, and 26 IU/L at baseline, day 7 and day 14, respectively.

Table 2 Serious adverse events, changes in creatinine and hemoglobin, and mortality.

Laboratory SAE[a] (grade)[b]	III	IV
Hypokalaemia	2	0
Elevated creatinine	4	0
Elevated ALT	0	0
Anaemia	0	0
Neutropenia	5	2
Thrombocytopenia	2	0
Laboratory parameter	*Day 7*	*Day 14*
Decrease in hemoglobin level, >2 g/d, n/N (%)	4/23 (17)	2/20 (10)
Decrease in hemoglobin level, % change, mean ± SD	8 ± 8	6 ± 8
Creatinine level >2 x baseline level n/N (%)	1/25 (4)	1/22 (4.5)
Increase in creatinine level, % change, mean ± SD	44 ± 37	24 ± 37
Deaths		
2 weeks (%)	7 (23%)	
10 weeks (%)	8 (28%)[c]	

[a] Clinical grade III/IV SAE not related to cryptococcal infection included pneumonia (2), tuberculosis (1), gastroenteritis (1), hemiparesis thought related toxoplasmosis (1).

[b] Cut-offs used to define DAIDS Grade III and Grade IV lab abnormalities: potassium (III 2.0—2.4 mmol/L; IV < 2.0 mmol/L), creatinine (laboratory range, male 0.6—1.1 mg/dl, female 0.5—0.9 mg/dl, III male >2.1—3.7 mg/dl, female >1.7—3.1 mg/dl; IV male >3.7 mg/dl, female >3.1 mg/dl), ALT/SGPT (III >175—350 IU/L; IV > 350 IU/L), hemoglobin (III 6.5—7.4 g/dl; IV < 6.5 g/dl), absolute neutrophil count (III 500—749/mm^3 IV < 500/mm^3), platelets (III 25,000—49,999/mm^3; IV <25,000/mm^3).

[c] 1 patient was lost to follow up.

Discussion

In this cohort of patients treated with a short, 5-day course of AmB, the rate of clearance of infection observed was more rapid than previously seen at the same centre with high dose fluconazole alone: In the previous cohorts studied at the same site,[9] the EFA was −0.08 ± 0.17 log CFU/d for fluconazole at 800 mg/d, and −0.18 ± 0.11 log CFU/d for fluconazole at 1200 mg/d, considerably less rapid than the EFA of −0.30 ± 0.11 log CFU/d with the current cohort. It should be emphasized that this was not a randomised comparison. Rather patients treated with fluconazole alone were historical comparative cohorts, and may have been differed from the current cohort in important respects, not assessed during our studies. However, the inclusion criteria and baseline characteristics were very similar; and adjusting for the small rise in presenting CD4 count in the current cohort, had no effect on estimates for the differences in rate of clearance between the cohorts.

The EFA for this cohort treated with 5 days of AmB was very similar whether calculated over the first 14 days or just the first 7 days. This continued monoexponential clearance over the full 14 days strongly suggests that the CFU counts would have been the same at day 14 even if the AmB dosing had been continued to day 14. The same was noted in a cohort treated with 7 days of AmB alone in Cape Town.[8] The observation is consistent with the long half-life of AmB, especially perhaps within tissues, and supports the concept that shorter courses of AMB *may* not have significantly reduced efficacy compared to the standard of 2 weeks. Presumably at some time after 14 days the effect

of short course AmB would diminish before that of standard 2 week AmB, but by that time infection should be under control. The EFA for this cohort was very similar to that measured for optimised oral therapy with high dose fluconazole plus flucytosine in Malawi (-0.28 ± 0.17 log CFU/d),[12] further supporting the equipoise of comparing this optimal oral regimen with AmB-based treatment.

Although the numbers are too small and the study was not powered to show mortality differences, the trend toward reduced mortality seen in this cohort (23 and 28% 2- and 10-week mortality) compared with the earlier cohort from Mbarara treated with fluconazole 1200 mg/d alone (22 and 48% 2- and 10-week mortality),[9] suggests the generally-accepted superiority of AmB-based treatment over fluconazole monotherapy[4,5,8,17,18] may hold even if the fluconazole is high dose. The outcomes also add further data suggesting no clear-cut difference in outcomes between 1-week[8] with standard 2-week[14,16,17,19] AmB induction, and support the need to compare 1-week with 2-week AmB induction in a larger trial in African centres.

AmB for 5 days was well-tolerated. AmB and high dose fluconazole were not temporarily held or stopped early in any patient because of side effects. With routine fluid and potassium supplementation, there was no grade IV hypokalemia, renal impairment, or anemia. The results are compatible with the predictable, dose-dependent nature of the common side effects of AmB. In comparison to this experience (summarised in Table 2), with 2 weeks of AmB in Cape Town,[14] at 0.7 and 1.0 mg/kg/d dosages of AmB, respectively: the mean percent fall in hemoglobin was 16% and 25%; hemoglogin fell by >2 g/dl in 50 and 71% of patients; and 13% and 32% of patients developed a creatinine level at 2 weeks greater than twice the baseline level.[14] The cohort also adds further data that supports the apparent safety of fluconazole at 1200 mg/d for the initial 2 weeks in this clinical setting.

The results suggest a short 5-day course of amphotericin B could be given with reasonable safety even if laboratory monitoring was not as reliable as is the case in a study setting. Given savings in duration of admission, drug costs, costs for laboratory monitoring, and fluid and electrolyte replacement costs, short course AmB (5–7 days) is also much more widely implementable than 2 week AmB induction in resource-restricted settings and where patients contribute to the costs of their care. In Mbarara University hospital, short course AmB has now been adopted as the standard of care.

Phase III, randomized controlled trials are warranted and planned in African centers to compare the efficacy of short course AmB-based treatment with a standard 2-week course of AmB, and with optimised oral therapy with high dose fluconazole plus flucytosine.[12] A more widely sustainable, safe alternative regimen that was as effective as the standard of 2 weeks AmB induction has the potential to substantially reduce the 10 week mortality of patients in centers still using fluconazole monotherapy from over 50%[7,9,12] to around 25–35%.[14–17]

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Potential conflict of interest

All authors: no conflicts.

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