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REVIEW ARTICLE

COMPARISON OF AMPHOTERICIN B AND ITS COMBINATION WITH FLUCY TOSINE IN THE MANAGEMENT OF CRYPTOCOCCAL MENINGITIS: A REVIEW

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ABSTRACT

Cryptococcosis is a global invasive mycosis that is associated with high morbidity and mortality. With its profound propensity to locate within the central nervous system, which is frequently accompanying by fungal meningitis. Immunocompromised patients with cryptococcal meningitis should receive Amphotericin B deoxycholate 0.7–1.0 mg/kg/day intravenously plus Flucytosine 100 mg/kg/day divided into four doses for at least 2 weeks, followed by Fluconazole, 400 mg orally daily, for a minimum of 8 weeks OR Amphotericin B monotherapy for 10 weeks duration. Hence, the objective of this review was to compile evidence on the comparison of the effectiveness of Amphotericin alone and combined with Flucytosine in the management of cryptococcal meningitis. A literature review of research articles was done, by accessing electronic journals from MEDLINE, EMBASE, COCHRANE LIBRARY and PUBMED published from years 1979 to 2018. Findings of randomized clinical trials studies done in English and published documents were included in the review. The available evidence from the included studies finding supported that combining Amphotericin B with Flucytosine had better clinal improvement than AmB monotherapy. In all studies included Flucytosine addition resulted in better outcomes in cerebrospinal fluid yeast count, serologic evidence, clinical symptoms, survival rate, or occurrence of adverse drug events.

INTRODUCTION

Cryptococcal meningitis (CM) is an infection of the brain parenchyma and subarachnoid space by the encapsulated saprophytic yeast organism, *Cryptococcus neoformans* (1). In many areas where there is a high prevalence of human immunodeficiency virus (HIV), such as sub-Saharan Africa, it has become the leading fatal adult meningitis (2). Although healthy hosts can be infrequently affected, the disease occurs frequently in immunosuppressed individuals. In HIV -positive patients, autoimmune deficiency syndrome (AIDS-associated CM) typically affects individuals with a CD4 cell count <100 cells/mm3 (3).

Cryptococcal meningitis is a common opportunistic infection and AIDS-defining illness in patients with late-stage HIV infection, particularly in Southeast Asia and Southern and East Africa (4). Although the extensive availability of antiretroviral therapy (ART) has substantially reduced the worldwide prevalence of Cryptococcosis, it has still become a major problem in developing countries. Early diagnosis and treatment are the keys to treatment success (5). It was predicted that in 2014 there were over 220,000 new cases of CM globally resulting in more than 180,000 deaths and responsible for15% of all AIDS-related deaths (6), which contributes up to 20% of AIDS-related deaths in low- middle-income countries per annum (7).

Cryptococcal meningitis was a steadily fatal disease before the introduction of Amphotericin B (AmB). Flucytosine (5-FC) can also cure this infection, but secondary drug resistance and a low proportion of cures make this drug unattractive as a single agent. In vitro and in vivo evidence suggested that AmB and 5-FC were additive in their effects against cryptococcus prompted clinical trials of the combination (8).

Immunocompromised patients with CM should receive AmB 0.7–1.0 mg/kg/day intravenously plus 5-FC 100 mg/kg/day divided into four doses for at least 2 weeks, followed by Fluconazole (FLU) 400 mg orally daily for a minimum of 8 weeks (9). For a setting where 5-FC is unavailable, a combination of AmB and high-dose FLU 800–1200 mg per day is used as second-line induction treatment. The initial 2 weeks induction treatment should be followed by consolidation and maintenance phases of treatment with FLU (10).

AmB was the first effective therapy and, at doses of 0.3–0.5 mg/kg/day for 10 weeks, led to cure rates of over 50% in non-HIV-associated infection. It has concentration-dependent activity, and more recent trials at doses of 0.7 mg/kg/day have yielded improved results (4). AmB given for 4–6 weeks has been considered to be the gold standard for the initial treatment of CM in patients with HIV infection (11).

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5-FC is the most common choice and is recommended in the CM treatment guidelines. However, this treatment has not been shown to reduce mortality, as compared with amphotericin B monotherapy. 5-FC is frequently unavailable where the disease burden is greatest, and concerns about cost and side effects have limited its use in resource-poor settings (7). Monotherapy with 5-FC commonly led to the development of resistance which is not a problem when it is combined with AmB. This combination has also been shown to have additive effects in both pre-clinical studies and clinical trials in non-HIV-associated and HIV-associated infections (4).

Although guidelines exist for the antifungal management of CM, recommendations are based on limited data from randomized controlled trials (RCTs), and in clinical practice, treatment is highly variable due to drug costs, availability, and ability to monitor and manage drug-related. A Cochrane review on treatment for HIV-associated cryptococcal meningitis was published in 2018 (12), but several clinical trials comparing new induction regimens in treating non-HIV associated had not been included. Therefore, this review aimed to compile evidence which compares AmB monotherapy versus a combination regimen of AmB with 5-FC as part of an induction regimen for cryptococcal meningitis.

METHODS

Search strategy and search terms

A systematic review of research articles from electronic journals including Medline, EMBASE, Cochrane Library, and PubMed was done, which were published from 1979 to 2018. The following keywords were used as search terms: cryptococcal meningitis, Cryptococcus neoformans, meningitis, amphotericin B, flucytosine, Amphotericin B with flucytosine, human immunodeficiency virus, and by connecting each of these keywords using Boolean operators.

Data extraction and Review system

We extracted key information by reference: including journal, title, author, volume in page numbers; objective: the study objective as stated by the authors; study design: randomized clinical trials; population: demographics of the participants in the study and outcome: results.

Earlier studies compared AmB and combined with 5-FC in treating CM, had been found to show variation in terms of outcomes, and also, they were conducted by different study designs. For this reason, in this review, we didn't compare and contrast each finding and the results were summarized according to the category of each separate study.

Inclusions and exclusion Criteria

Findings from RCTs articles, studies done in English, published from 1979 to 2018 were taken as inclusion criteria. However, journals with abstract only, and written in languages other than English were not included in the review.

Quality of review

Quality evaluation was performed in which each article received a summary quality measurement that corresponded with important attributes of the study, adapted from previous studies. These include research design, data analysis, measurement and validity, the strength of the study and, consistency or homogeneity of the findings across the studies.

Types of outcome measures

The primary treatment outcome measures in this review include cerebrospinal fluid (CSF) yeast count, serologic evidence, clinical symptoms, mortality, whereas drug-related adverse events and rate of fungal clearance were considered as secondary outcome measures.

RESULTS

A total of 76 journal articles were downloaded, of which 68 were excluded due to various reasons. Finally, 8 articles were selected for eligibility. The search and selection strategy is presented as a PRISMA flow chart in figure 1.

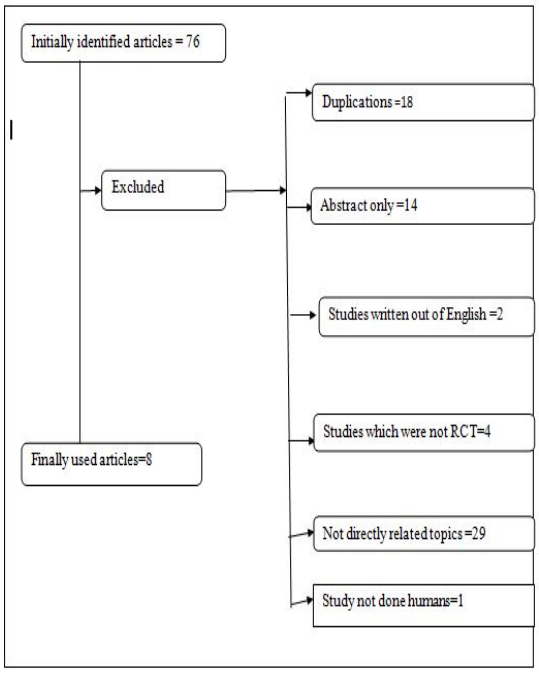

Figure 1: Flow chart showing selection process of articles in systematic review.

The review covered studies conducted to evaluate the efficacy of AmB versus AmB plus 5-FC after 14, 28, 42, and 70 days of treatment. Of the included studies, five of them compared AmB monotherapy versus AmB plus 5-FC, one study evaluated the efficacy of AmB monotherapy, another one study evaluated the efficacy of AmB plus 5-FC combination regimen and the rest one study compared AmB plus 5-FC combination regimens at different doses of AmB (Table 1).

Table 1: Comparison of Amphotericin and combined with Flucytosine in treatment of cryptococcal meningitis

Study	Name and dose of drugs	Sample size	Evaluation day	Primary outcomes	Improved patients (%)	Death (%)
(Day et al.,2013) (10)	AmB-1m/kg/day	99	14 days	CSF yeast count and clinical symptoms*	74 (74.75)	25 (25.3)
	AmB 1m/kg/day +5 -FC 100mg/kg/day	100	14 days		85 (85)	15(15)
(Dismukes, 1987) (8)	AmB-0.4mg/kg/day	27	70 days	CSF culture♠and clinical symptoms*	11(40.74)	5 (18.52)
	AmB-0.3mg/kg/ day+5-FC-150mg/ kg/day	24	42 days		16(66.67)	5 (20.83)
(Perfect et al., 2010) (13)	AmB-0.7mg/kg/ day+5-FC-100mg/ kg/day	30	70 days	CSF yeast count and serologic evidences	24(80)	6(20)
	AmB-1mg/kg/ day+5-FC-100mg/ kg/day	34	70 days		25(73.53)	8 (23.53)
(Jackson et al., 2012) (9)	AmB-0.3mg/kg/ day+5-FC-150mg/ kg/day	202	14 days	CSF culture♠ and clinical symptoms*	102(50.5)	11 (5.44)
	AmB-1m/kg/day	179	14 days		76(42.46)	10 (5.59)
(Molloy et al., 2018) (14)	AmB-1mg/kg/ day+5-FC-100mg/ kg/day	228	70 days	CSF yeast count, clinical symptoms*	157(68.8)	71 (31.2)
	AmB-1m/kg/day	229	70 days		138(60.2)	91 (39.7)
(Bicanic et al., 2008) (15)	AmB-1m/kg/day	48	70 days	CSF culture♠	32 (66.6)	16 (33.3)
(Dromer et al., 2008) (16)	AmB-1mg/kg/ day+5-FC-100mg/ kg/day	142	14 days	CSF culture♠	51 (36)	No report
	AmB-1m/kg/day	142	14 days	CSF culture♠	89 (63)	
(Loyse et al., 2012) (17)	AmB-0.7mg/kg/ day+5-FC-100mg/ kg/day	80	70 days	CSF culture♠	57(71.25)	23 (28.8)

**The clinical outcome was considered to be successful if fever, headache, and meningismus were improved or no worse*

♠- Mycological outcome was considered to be successful if CSF fungal culture was negative, CSF-cerebrospinal fluid, AmB-Amphotericin B,5-FC-Flucytosine

Outcomes-based on the evaluation period

On 2nd week

Day *et al.*, 2012 reported that there is no significant differences in survival rates across groups at day 14 (15 deaths in patients treated with AmB 1m/kg/day +5 -FC 100mg/kg/day vs 25 deaths in patients who took AmB-1m/kg/day; p=0.08) (10). Molloy *et al.*, 2018 also revealed that there were no significant differences in the proportions of patients with stable or improved symptoms (78% of the combination-therapy group and 83% of the AmB group, P=0.18), unchanged or improved (77% of the combination-therapy group and 74% of the AmB group, P=0.42), or combined mycological and clinical responses (50% of the combination-therapy group and 42% of the AmB group, p=0.12) (14).

Results from a cohort study with 208 HIV-positive and negative patients with meningoencephalitis clearly emphasized the success of therapy with AmB plus 5-FC for 14 days over any other induction regimen in persons with high fungal burden disease and abnormal neurological features, showing that there was a 26% failure rate in the combination group versus a 56% failure rate for other treatments (P <0.001) (13).

On 6th week

Dismukes *et al*., 1987 compared a combination of AmB and 5-FC versus AmB monotherapy in 66 patients. The result showed that 23 of 34 (68%) were cured or improved by combination, and 15 of 32 (47%) by AmB monotherapy (p>0.05). Similar results were also reported when courses not adhered to the protocol were excluded totally; 16 of 24 patients (67%) were cured or improved by the combination, and 11 of 27 (41%) by AmB alone (x^2=2.47, p> 0.05) (8).

At 10th week

Bicanic and Harrison, 2005 showed mortality rate was 24% (15 of 63 patients) at 10 weeks, with no difference between groups (AmB-0.7mg/kg/day+5-FC-100mg/kg/day vs. AmB-1mg/kg/day+5-FC-100mg/kg/day). They also illustrated that 68% and 60% of patients were alive at 6 months and 1 year, respectively and there was no difference in survival rates between the 2 groups at any time point (4). Day *et al.*, 2013 also exposed that by day 70, a total of 44 patients treated with amphotericin B monotherapy had died, as compared with 30 patients treated with AmB and 5-FC, in which treatment with a combination regimen is associated with a significantly reduced hazard of death by day 70 in the intention-to-treat analysis (hazard ratio, 0.61; 95% confidence interval (CI), 0.39 to 0.97; P=0.04) (10).

A study conducted by Molloy et al. also compared AmB plus 5-FC versus AmB plus FLU combination regimens for the management of CM. This study revealed that AmB plus 5-FC was superior to AmB plus FLU (71 deaths (31.1%) vs. 101 deaths (45.0%); hazard ratio for death at 10 weeks, 0.62; 95% confidence interval (CI), 0.45 to 0.84; P=0.002) (14).

DISCUSSION

Patients treated with combination of Amphotericin B with Flucytosine at doses of AmB-1m/kg/day+5-FC-100mg/kg/day and AmB-0.3mg/kg/day+5-FC-150mg/kg/day, after 14 days of initiating therapy, showed greater improvements, i.e. 85% and 50.5%, respectively (10,14) than those treated with AmB 1mg/kg/day alone, which was 42.46% (14).This finding aligns with guidelines of the Infectious Disease Society of America (14) which recommend the first choice for induction-phase treatment as: AmB (0.7 - 1.0 mg/kg/dose) plus 5-FC (100 mg/kg/day) (10). A regimen of 0.7 – 1.0 mg/kg q24h in combination with 5-FC for two weeks is currently recommended for induction therapy (18).

The rate of clearance of infection during the first 2 weeks of therapy was more rapid for group 2 (AmB-1mg/kg/day+5-FC-100mg/kg/day) than for group 1 (AmB-0.7mg/kg/day+5-FC 100mg/kg/day). The mean early fungicidal activity (SD) was -0.56 +/- 0.24 log cfu/mL of CSF per day for group 2 and -0.45 +/- 0.16 log cfu/mL of CSF per day for group 1 (15). Dromer *et al.*, 2008 reported that mycological failure at week 2 was significantly less frequent among patients treated with AmB+5-FC than AmB monotherapy, which was 20/86 (23%) vs. 47/100 (47%) (16).

It also revealed that with the same yield of sterilization for patients with meningoencephalitis, even those with abnormal neurology at baseline, the highest rate of mycological failure was observed for AmB alone. Mortality was lower in patients who were given AmB and 5- FC at the 3 months point but the overall reduction in mortality with the 5-FC combination group was not different (2).

Even though the combination regimen was given for only six weeks and monotherapy of AmB for 10 weeks, the combination cured or improved more patients (16 vs. 11), produced fewer failures or relapses (3 vs. 11), more rapid sterilization of CSF (p<0.001) and less nephrotoxicity (p<0.05) than did AmB monotherapy (19).

But, Molloy *et al.*, 2018 reported that 11 patients (2.9%) had toxic effects requiring the withdrawal of the study drug (6 receiving combination therapy and 5 receiving amphotericin B alone). Three patients had elevated serum creatinine values, two had nausea, two had hypokalemia, and one each had a rash, headache, hemolytic anemia, and a gastrointestinal hemorrhage (14).

Day *et al.*, 2013 also reported that by day 70, a total of 44 (total patients =99) patients treated with AmB monotherapy had died, while 30 patients treated with AmB plus 5-FC and 33 patients treated with AmB plus FLU. It revealed that treatment with AmB plus 5-FC was associated with a drastically reduced hazard of death by day 70 in the intention-to-treat analysis (hazard ratio, 0.61) (10). AmB plus 5-FC has also shown to be the most potent and advocated regimen for the induction phase (5).

The time to fungal clearance was significantly shorter in patients receiving AmB plus 5-FC than in those receiving AmB monotherapy, with more rapid rates of decline in the colony count (−0.42 log10 CFU per day vs. −0.31 log10 CFU per day) (10). However, on day 70, a visual deficit was reported in 16 of 46 assessed patients treated with AmB, as compared with 9 of 54 patients treated with AmB plus 5-FC. Neutropenia was more frequent among patients receiving AmB plus 5-FC than those receiving amphotericin B monotherapy (34% vs. 19%).

Surprisingly, a Phase II Randomized Controlled Trial conducted in Malawi showed that early fungicidal activity (EFA) for the triple combination of AmB plus FLU plus 5-FC was greater than for AmB plus FLU: -0.50 ± 0.15 log CFU/day vs. -0.38 ± 0.19 log CFU/day (p = 0.03); and greater than FLU plus 5-FC (-0.28 ± 0.17) or FLU alone (-0.11 ± 0.09). Combined analysis across steps revealed that addition of 5-FC plus AmB had significant, independent additive effects on EFA, with trends toward fewer early deaths with the addition of 5-FC (4/41 vs. 11/39, p = 0.05) and fewer deaths overall with the addition of AmB (13/39 vs. 20/40, p = 0.1) (20).

A retrospective cohort study done at Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China revealed that a combination of FLU plus 5-FC is promising in treating non-HIV- and non-transplant-associated CM patients who do not tolerate or are not suited for AmB plus 5-FC regimen. There is no significant differences in cryptococcus clearance (74.4% vs 70.2%, $P = 0.814$), treatment time (39 days, 20-69 days vs 21 days, 7-63 days, $P = 0.107$) and successful response rates (69.7% vs 72.3%, $P = 0.820$), but FLU plus 5-FC treatment had lower total adverse events (19.1% vs 90.7%, $P < 0.001$) (21).

Limitation of the review

This review excludes studies done in languages other than English and abstracts only pieces of the manuscripts. The literature review was done on the effectiveness of Amphotericin B alone and combining Amphotericin B with Flucytosine which has wide variations regarding strength. There was a lack of recent randomized clinical trials comparing the two regimens.

The cure rates we encountered also cannot be compared easily with various reports because the characteristics of patients affect the outcome.

Conclusion and Recommendations

Our review indicated that combining Amphotericin B with Flucytosine showed better sterilization of cerebrospinal fluid and improvement of clinical symptoms which leads to the conclusion that a combination regimen is better than Amphotericin B monotherapy of Cryptococcal meningitis. Further studies should be done to include studies done in languages other than English. A continuation arm of the interim analysis reports of some of the study should be incorporated to answer some of the above limitations to increase updated literature sources. Assessment of compliance with the prescribed drug therapy, which may have had an impact on effectiveness outcomes, should also be done.

Competing of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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