AmphotericinB and Fluorocytosine Combined with Voriconazole for the Treatment of Non-HIV- and Non-Transplant-Associated Cryptococcal Meningitis: A Retrospective Study

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Abstract

Background

This retrospective study aimed to explore the curative effect of amphotericin B (AMB) and fluorocytosine (5-FC) combined with voriconazole (VOR) in the induction period for the treatment of non-HIV- and non-transplant-associated cryptococcal meningitis (CM).

Methods

Clinical data from patients admitted to the Third Affiliated Hospital of Sun Yat Sen University from January 2011 to December 2020 were collected. The patients were stratified based on antifungal treatment methods in the induction period (group I with AMB + 5-FC + VOR, group II with AMB + 5-FC + FLU, group III with AMB + 5-FC).

Results

The length of hospital stay in group I was significantly shorter than those in group II ($p = 0.000$) and group III ($p = 0.000$). Among the patients who achieved CSF sterility within 10 weeks, the number of days to reach CSF sterility in group I was shorter than that in group II ($p = 0.046$). The incidence of hypokalemia in group I was significantly lower than those in group II ($p = 0.003$) and group III ($p = 0.004$). The incidence of gastrointestinal discomfort in group I was significantly lower than that in group II ($p = 0.004$).

Conclusion

AMB plus 5-FC combined with VOR for the induction treatment of non-HIV- and non-transplant-associated cryptococcal meningitis is a better choice than AMB plus 5-FC combined with FLU and AMB plus 5-FC. This new triple antifungal treatment method rapidly cleared the cryptococci from the CSF, substantially shortened the hospital stay, and decreased the incidences of hypokalemia and gastrointestinal discomfort.

Background

Cryptococcal meningitis (CM) is a severe disease that has high morbidity and mortality[1, 2]. Due to the emergence of highly active antiretroviral therapy (HAART), the morbidity and mortality of HIV-associated CM has gradually decrease[3]. However, the number of patients with non-HIV-associated CM has increased in recent years[4–6]. Recently, some studies showed that compared with HIV-infected individuals, non-HIV-positive CM patients had a higher mortality rate [7, 8]. Therefore, antifungal treatment for non-HIV- and non-transplant-associated CM should be given more attention.

Currently, antifungal treatment for CM is divided into three phases: induction therapy, consolidation therapy, and maintenance therapy. Induction therapy is considered to be the most crucial phase of the antifungal treatment for CM. Induction therapy with amphotericin B (AmB) combined with 5-flucytosine (5-FC) at subtherapeutic doses for at least 4 weeks is recognized as the preferred regimen for non-HIV-infected and nontransplant patients with CM worldwide [9]. However, this preferred regimen is still associated with mortality rates of 15–40% [10, 11]. The probable reason is that many CM patients cannot endure the recommended dosages of antifungal drugs due to their severe toxic effects. In response to this phenomenon, Xu et al. invented a triple antifungal method (AMB and 5-FC at subtherapeutic doses combined with FLU), which was shown to be better than the previous double antifungal therapy in the induction period for non-HIV-infected and nontransplant patients with CM in a Chinese tertiary hospital [12]. However, due to the continuous emergence of fluconazole resistance, the efficacy of triple antifungal therapy for CM is worsening [13].

Voriconazole (VOR) is a second-generation azole antifungal agent. It is more potent than fluconazole (Flu) against fungal P-450-dependent 14 α -sterol demethylase and more effective than fluconazole at penetrating fungal cells [14]. Recently, some case reports showed that VOR could be used as an alternative treatment in CM patients in whom standardized treatment had failed [15–17]. Therefore, we compared the efficacy of combination therapies among double antifungal therapy (AMB and 5-FC), traditional triple antifungal therapy (AMB and 5-FC combined with FLU), and the new triple antifungal therapy (AMB and 5-FC combined with VOR) in our study.

Methods

Patients and definitions

This retrospective study collected data from all non-HIV- and non-transplant-associated CM patients who were admitted to the Third Affiliated Hospital of Sun Yat-Sen University in Guangzhou, China, from Jan 2011 to Dec 2020. The diagnosis was confirmed based on symptoms and signs that were consistent with CM and positive results of India ink staining of the CSF or CSF cultures for *Cryptococcus neoformans* [18]. Patients meeting all the following criteria were included: (1) CM was first diagnosed in our hospital; (2) the antifungal therapy plan in the induction period was AMB + 5-FC, AMB + 5-FC + FLU, or AMB + 5-FC + VOR; and (3) induction therapy was administered for more than 2 weeks. Patients meeting any of the following criteria were excluded: (1) the antifungal therapy plan was changed in the induction period; (2) a surgical intervention had previously been performed due to intracranial hypertension; (3) no follow-up visit within 10 weeks; and (4) recurrent CM. A total of 63 non-HIV- and non-transplant-associated CM patients were recruited and further divided into three groups according to the treatment regimens (15 patients in group I received AMB + 5-FC + VOR, 30 patients in group II received AMB + 5-FC + FLU, and 18 patients in group III received AMB + 5-FC). The details of the inclusion and exclusion of patients are shown in Fig. 1. (Fig. 1)

Clinical manifestation assessment

The British Medical Research Council (BMRC) staging system is an appropriate method of assessing CNS symptom severity. These systems classify CNS symptoms into three stages according to severity: stage 1, normal sensorium with no focal neurological deficit; stage 2, slight or no neurological deficit (cranial nerve palsy) and/or mild clouding of the sensorium; and stage 3, severe focal neurological deficit (multiple cranial nerve palsies), severe impairment of sensorium, convulsions, and/or involuntary movement [19]. The evaluation was performed and compared at discharge and after 10 weeks of antifungal treatment.

Laboratory examinations

The enrolled patients underwent lumbar punctures at least once a week in accordance with the guidelines [9, 20]. The CSF open pressure; CSF white blood cell count; glucose, chloride, and protein levels; and India ink staining results were recorded. The CSF burden of cryptococcal organisms was evaluated by India ink staining and CSF cryptococcal organism counts.

Therapeutic regimens

The details of the therapeutic regimens in the three groups are presented in Table 1. AMB, 5-FC, VOR, and FLU were administered concurrently each day by intravenous drip for at least 2 weeks. The maximum tolerated dosages of AMB or 5-FC in the different groups were not significantly different ($p > 0.05$).

Table 1
Therapeutic regimens in three groups

The maximum tolerated dosages of AMB or 5-FC and the average dosages of VOR or FLU	Group I (AMB + VOR + 5-FC, n = 15)	Group II (AMB + FLU + 5-FC, n = 30)	Group III (AMB + 5-FC, n = 18)	P-value
AMB (mean $\pm$ SD), mg/kg	0.69 $\pm$ 0.11	0.62 $\pm$ 0.13	0.58 $\pm$ 0.15	0.074
5-FC (med, range), mg/kg	95.24 (53.33-129.17)	84.52 (56.34-150.00)	72.08 (43.48-146.34)	0.123
VOR or FLU (med, range), mg/d	400 (400-600)	600 (400-800)	NA	NA

AMB amphotericin B, 5-FC flcytosine, VOR voriconazole, FLU fluconazole, NA not available. Data were presented as the mean $\pm$ SD, median (range). Continuous variables were analyzed by one-way ANOVA or kruskal wallis H test. * $P < 0.05$.

Outcome assessments

The treatment response evaluated at the 10th week after the initiation of antifungal therapy in each patient was recorded. The therapeutic outcomes were classified into five levels: (1) complete response: survival and resolution of all attributable symptoms and signs of disease with CSF clearance; (2) partial response: survival and CSF clearance with the persistence of attributable symptoms and signs of disease; (3) stable response: survival with minor or no improvement in attributable symptoms and signs of disease and persistently positive CSF culture results; (4) disease progression: worsening clinical

disease symptoms or signs and persistently positive CSF culture results; and (5) death: death during the prespecified evaluation period, regardless of cause[21]. CSF clearance was defined as negative CSF cryptococcal organism culture and no CSF cryptococcal organisms observed on India ink staining.

Statistical analysis

Baseline demographic and clinical characteristics are presented as percentages, means with standard deviations (SD), or medians with ranges. Comparisons were performed using chi-square tests or Fisher's exact tests for categorical data and with one-way ANOVA or Kruskal-Wallis H tests for continuous data. The efficacy of treatment was estimated using the chi-square test based on the five grades over the ten weeks among the three study groups. Chi-square tests were used to compare CSF sterility within 2, 4, and 10 weeks. Chi-square and Fisher's exact tests were used to compare the incidences of adverse events among the three groups. Statistical analyses were performed using SPSS statistics version 25 (IBM). All analyses were two-sided, and P-values < 0.05 were considered statistically significant.

Results

Baseline patient characteristics

The details of the baseline patient characteristics are presented in Table 2. The length of hospital stay in group I was significantly shorter than those in group II ($p = 0.000$) and group III ($p = 0.000$). However, the length of hospital stay between group II and group III was not significantly different ($p = 1.000$). There were no significant differences among the three groups in other baseline characteristics.

Table 2
Baseline patient characteristics.

Variables	Group I (AMB + VOR + 5-FC, n = 15)	Group II (AMB + FLU + 5-FC, n = 30)	Group III (AMB + 5-FC, n = 18)	P-value
Age, years (mean $\pm$ SD)	44.47 $\pm$ 17.32	41.13 $\pm$ 13.09	48.11 $\pm$ 12.09	0.248
Gender, male (n, %)	9 (60.0%)	19 (63.3%)	13 (72.2%)	0.735
Length of hospital stay days (med, range)	23.00 (14–43)	54.00 (23–119)	71.00 (16–224)	0.000*
BMRC staging (n, %)	0 (0%)	2 (6.7%)	0 (0%)	0.312
1	15 (100%)	26 (86.7%)	15 (83.3%)	0.279
2	0 (0%)	2 (6.7%)	3 (16.7%)	0.288
3	7 (46.7%)	20 (66.7%)	13 (72.2%)	0.273
CSF parameters	123.00 (14–350)	105.50 (14–740)	95.00 (10–300)	0.179
Opening pressure $\geq$ 200 mmH ₂ O (n, %)	13 (86.7%)	23 (76.7%)	11 (61.1%)	0.089
CSF WBC count $\times 10^6$ /l (med, range)	7 (46.7%)	22 (73.3%)	10 (55.6%)	
CSF protein g/l (med, range) $\geq$ 0.45 (n, %)	24 (0–80500)	1561 (0–30000)	750 (0–14000)	
CSF glucose mmol/l (mean $\pm$ SD) $\leq$ 2.2 (n, %)				
CSF cryptococci count/ml (med, range)				

AMB amphotericin B, 5-FC flucytosine, VOR voriconazole, FLU fluconazole, WBC white blood cell, SD standard deviation, med median, CSF cerebrospinal fluid, BMRC British Medical Research Council. Data were presented as the mean $\pm$ SD, median (range) or n (%). Continuous variables were analyzed by one-way ANOVA or Kruskal Wallis H test; categorical variables were analyzed by Chi-square test or Fisher's exact test. *P < 0.05.

Outcome of clinical manifestations

The outcomes of the clinical manifestations were judged by comparing the BMRC stage for each patient at admission and at 10 weeks of antifungal treatment. There were no significant differences among group I, group II, and group III. The details are presented in Table 3.

Table 3

The change in neurological function circumstances evaluated by comparing the BMRC staging between patients at admission and at 10 weeks of follow-up.

BMRC	Group I (AMB + VOR + 5-FC, n = 15)	Group II (AMB + FLU + 5-FC, n = 30)	Group III (AMB + 5-FC, n = 18)	P-value
Improved(n,%)	11(73.3%)	16(53.3%)	15(83.3%)	0.084
Unchanged(n,%)	4(26.7%)	12(40.0%)	3(16.7%)	0.226
Worse(n,%)	0(0%)	2(6.7%)	0(0%)	0.493
AMB amphotericin B, 5-FC flcytosine, VOR voriconazole, FLU fluconazole. Data were presented as the n (%). categorical variables were analyzed by Chi-square test or Fisher's exact test. *P < 0.05.				

CSF sterility

The details of CSF sterility are presented in Table 4. The achievement of CSF sterility within 10 weeks was not significantly different ($p = 1.000$) among the three groups. However, among the patients who achieved CSF sterility within 10 weeks, the duration from the initiation of treatment to CSF sterility did significantly differ ($p = 0.032$). The number of days to reach CSF sterility in group I was significantly shorter than that in group II ($p = 0.046$), but there was no significant difference between group I and group III ($p = 0.069$). There was also no significant difference between group II and group III ($p = 1.000$). In addition, the patients who achieved CSF sterility within 2 weeks and 4 weeks among these three groups were not significantly different ($p^{2\text{weeks}} = 0.114$, $p^{4\text{weeks}} = 0.068$).

Table 4

CSF sterility at 10 weeks after the initiation of antifungal treatment.

	Group I (AMB + VOR + 5-FC, n = 15)	Group II (AMB + FLU + 5-FC, n = 30)	Group III (AMB + 5-FC, n = 18)	P-value
Case(n,%)	11(73.3%)	22(73.3%)	13(72.2%)	1.000
Days(med,range)	7(0–19)	23(0–69)	20(0–70)	0.032*
≤ 2 weeks(n,%)	8(53.3%)	7(23.3%)	5(27.8%)	0.114
> 2 weeks(n,%)	3(20.0%)	15(50.4%)	8(44.4%)	0.068
≤ 4 weeks(n,%)	11(73.3%)	14(46.7%)	6(33.3%)	
> 4 weeks(n,%)	0(0.0%)	8(26.6%)	7(38.9%)	
AMB amphotericin B, 5-FC flcytosine, VOR voriconazole, FLU fluconazole, med median,. Data are presented as the median (range) or n (%). Continuous variables were analyzed by kruskal wallis H test; categorical variables were analyzed by Chi-square test or Fisher's exact test. *P < 0.05.				

Treatment response

The primary treatment response outcome was evaluated at the 10th week after the initiation of therapy. No significant difference was observed in the treatment response rate, as shown in Table 5.

Table 5
Treatment outcomes based on induction therapy.

Treatment outcomes	Group I (AMB + VOR + 5-FC, n = 15)	Group II (AMB + FLU + 5-FC, n = 30)	Group III (AMB + 5-FC, n = 18)	P-value
Complete response(n,%)	8(53.3%)	14(46.7%)	11(61.1%)	0.622
Partial response(n,%)	3(20.0%)	6(20.0%)	2(11.1%)	0.757
Stable response(n,%)	4(26.7%)	8(26.7%)	5(27.8%)	0.709
Disease progression(n,%)	0(0%)	1(3.3%)	0(0%)	1.000
Death(n,%)	0(0%)	1(3.3%)	0(0%)	1.000

AMB amphotericin B, 5-FC flcytosine, VOR voriconazole, FLU fluconazole. Data were presented as the n (%). categorical variables were analyzed by Chi-square test or Fisher's exact test. *P < 0.05.

Adverse events

Most patients had common adverse events (such as chills and fevers, liver impairment, and renal impairment) during the 10 weeks of treatment (Table 6). The most common adverse events were renal impairment in group I (11/15, 73.3%) and hypokalemia in group II (27/30, 90.0%) and group III (17/18, 94.4%). The incidence of hypokalemia was significantly different among these three groups ($p = 0.001$). Patients in group I had a significantly lower incidence of hypokalemia than those in group II ($p = 0.003$) and group III ($p = 0.004$). In addition, the incidence of gastrointestinal discomfort among these three groups was significantly different ($p = 0.008$). By pairwise comparison, patients in group I had a significantly lower incidence of gastrointestinal discomfort than those in group II ($p = 0.004$). There was no significant difference between group I and group III ($p = 0.346$). There was also no significant difference between group II and group III ($p = 0.057$). The incidences of other adverse events were not significantly different among these three groups.

Table 6
Adverse events in patients with non-HIV- and non-transplant-associated CM.

	Group A (AMB + VOR + 5-FC, n = 15)	Group B (AMB + FLU + 5-FC, n = 30)	Group C (AMB + 5-FC, n = 18)	P-value
Adverse events(n,%)	15(100%)	30(100%)	18(100%)	NA
Chills and fevers(n,%)	0(3.4%)	2(6.7%)	2(11.1%)	0.565
Hypokalaemia(n,%)	7(46.7%)	27(90.0%)	17(94.4%)	0.001*
Gastrointestinal discomfort(n,%)	1(6.7%)	15(50.0%)	4(22.2%)	0.008*
Liver impairment(n,%)	6(40.0%)	22(73.3%)	12(66.7%)	0.088
Renal impairment(n,%)	11(73.3%)	19(63.3%)	7(38.9%)	0.105
Haematological impairment(n,%)	4(26.7%)	14(46.7%)	8(44.4%)	0.416
Visual side effects(n,%)	1(6.7%)	0(0%)	0(0%)	0.238
AMB amphotericin B, 5-FC flucytosine, VOR voriconazole, FLU fluconazole, NA not available. Data were presented as the n (%). Categorical variables were analyzed by Chi-square test or Fisher's exact test. *P < 0.05.				

Discussion

Recently, a network meta-analysis showed that AmB + 5-FC + Azole was superior to all other investigated induction regimens in HIV-positive CM patients. Previously, a retrospective study also confirmed that traditional triple antifungal therapy was superior to double fungal therapy as the induction regimen in non-HIV-positive CM patients in a Chinese tertiary hospital[12, 22]. The reasons we chose the new triple therapy instead of the traditional triple therapy were as follows: first, with the extensive use of fluconazole, resistance is increasing, and second, voriconazole has been shown to be more potent and effective than fluconazole *in vitro* and in animal models [14, 23–25].

In our study, we compared three antifungal therapies (new triple therapy, traditional triple therapy and double therapy) for the treatment of CM in the induction period. Although there was no statistically significant difference among these three groups in terms of the clinical manifestation and treatment response, the new triple therapy had 3 obvious advantages: first, it substantially shortened the hospital stay; second, it rapidly cleared the cryptococci from the CSF; and third, it decreased the incidence of some adverse events (hypokalemia and gastrointestinal discomfort) associated with antifungal drugs.

The clearance of cryptococci from the CSF is an important predictive factor for the prognosis of CM, and a slow rate of clearance of cryptococci was found to be independently associated with increased

mortality at 2 and 10 weeks[26]. The patients who received new triple therapy achieved CSF sterility earlier than the patients who received traditional therapy and double therapy and consequently had a shorter hospital stay.

AMB is an antifungal drug that has many adverse effects, such as hypokalemia and gastrointestinal discomfort[27]. Hypokalemia caused by amphotericin B is dose-dependent[28]. In our study, the new triple therapy obviously shortened the duration of hospitalization in non-HIV- and non-transplant-associated CM patients, and patients who received the new triple treatment had shorter durations of intravenous AMB treatment, which reduced the total dose of AMB. Therefore, the incidence of hypokalemia in the patients who received the new triple therapy was lower than those in the patients who received the traditional triple therapy and the patients who received the double therapy.

Unexpectedly, the improvement in neurological function and the clearance of cryptococci from CSF were not significantly different between groups II and III. These results are in contrast to those of a previous study in a Chinese tertiary hospital [12]. There are two possible reasons for these differences: (1) the sources of the enrolled cases were not identical (one was from the Third Affiliated Hospital of Sun Yat Sen University, and the other was from the Third Affiliated Hospital of Sun Yat Sen University and Jiangxi Chest Hospital); (2) the time spans of patient inclusion were different (one was from January 2011 to December 2020, and the other was from January 2006 to December 2014).

There were some limitations of our study. First, our study was a retrospective study, which meant that it was prone to produce selection bias and recall bias. Second, our data were obtained from a single center with a relatively small sample size. Consequently, a multicenter study with a larger sample size is needed in the future.

Conclusion

We hypothesis that amphotericin B and fluorocytosine combined with voriconazole for the induction treatment of non-HIV- and non-transplant-associated cryptococcal meningitis was a better choice than amphotericin B and fluorocytosine combined with fluconazole and amphotericin B plus fluorocytosine. This new triple antifungal method rapidly cleared the cryptococci from the CSF and substantially shortened the hospitalization duration. In addition, this new triple therapy decreased the incidence of some adverse events (hypokalemia, gastrointestinal discomfort) associated with antifungal treatment.

Abbreviations

CM

cryptococcal meningitis; AMB:amphotericin B; 5-FC:flucytosine; FLU:fluconazole; VOR:voriconazole; CSF:cerebrospinal fluid

Declarations

Ethics approval and consent to participate: All procedures performed in studies involving the patient included were in accordance with the ethical standards of the institutional board and with the 1964 Helsinki Declaration. The study was approved by the Medical Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen University. Informed consent was obtained from all individual participants included in the study.

Consent for publication: We have obtained consent to publish from the participant to report individual patient data.

Availability of data and material: The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests

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Authors' contributions: Y.J. and F.P. was involved in the literature review, planning and writing of the manuscript. JY.L., J.L., X.S., L.Y., A.W., X.X., M.L. and Y.W. collected the data. JY.L., J.L. and X. S. analyzed the data. JY.L. and J.L. prepared Tables 1-6. X.S. prepared Figure 1. Y.J., F.P., JY.L. were involved in writing and editing of the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Flow diagram of patient inclusion and exclusion.