ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 8, Issue 2, 2015

ISSN - 0974-2441

Review Article

CRYPTOCOCCOSIS IN ADVANCE STATE OF AIDS AND ITS CHALLENGING THERAPEUTIC REGIMEN: IS IT REALLY CURABLE?

SWETASRI BHATTACHARYA[1,2]*, ANINDYA BANERJEE[2,3]

[1]Department of Pharmaceutics, Himlayan Pharmacy Institute, Majitar, Sikkim, India. [2]Department of Pharmacology, Gupta College of Technological Sciences, Asansol, West Bengal, India. [3]Department of Pharmacology, Nilratan Sircar Medical College & Hospital, Kolkata, West Bengal, India. Email: anindyabanerjee1988@gmail.com

Received: 11 January 2015, Revised and Accepted: 03 February 2015

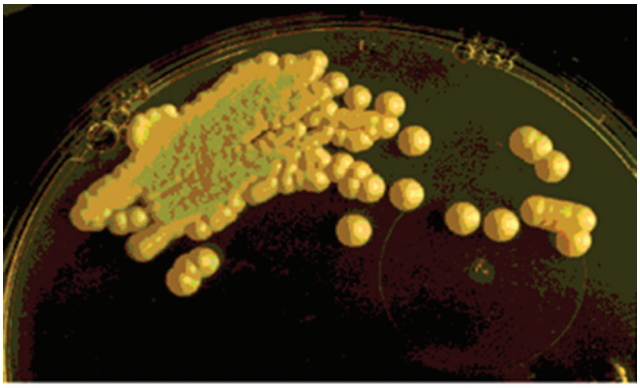
ABSTRACT

Cryptococcosis followed by *Cryptococcal meningitis* (CM) is a deadly disease in an immuno-compromised patient with the complication of advance human immunodeficiency virus/AIDS, which comes through an air born virus *Cryptococcus neoformans*, from bird dropping. Immune reconstitution inflammatory syndrome is also often associated with this disease. A 10 years study showed a 17% prevalence of CM in a tertiary care hospital, among which 53.1% were infected with AIDS. Treatment guideline is amphotericin B (AmB) and 5-flucytosine or AmB and fluconazole, but the iatrogenic effect, cost and scarcity of availability of these drugs are the leading cause of mortality due to this disease. Anyhow, various novel therapeutic approaches have been taken to reduce and/or to prevent the pathogenesis of CM for the sake of mankind.

Keywords: Antiretroviral therapy, Seizures, Hypokalemia, Hypomagnisemia.

INTRODUCTION

Cryptococcal meningitis (CM), the most common life-threatening opportunistic infectious disease. Patients are infected with the human immunodeficiency virus (HIV) Type 1, occurs in 5-8% of patients with the acquired immunodeficiency syndrome [1]. This disease is caused by two types of fungus: *Cryptococcus neoformans, Cryptococcus gatti*. Most of the cases, the incidence of infections caused by the encapsulated yeast *C. neoformans* has risen markedly over the past 20 years as a result of the HIV epidemic and increasing use of immunosuppressive therapies [2].



Cryptococcus neoformans on Sabouraud's agar
Culture of Cryptococcus neoformans on Sabouraud's agar.
Courtesy of Harriet Provine.

The most common disease CM, with over 1 million cases and 600,000 deaths/year. Non-menigeal (e.g. pulmonary and cutaneous) presentations also occur, bloodstream infection (cryptococcemia) may disseminate to multiple sites. The largest influence on the epidemiology of Cryptococcal disease over the last 30 years has been the evolution of the HIV pandemic. Well-recognized that sub-Saharan Africa has been the global region heavily affected by HIV, with an estimated 2.6 million new infections per year at the peak of the epidemic in 1997. Contemporaneously in the 1990 CM became the leading cause of adult meningitis in many African countries, including Malawai, Zimbabwe, South Africa. Clinical trials estimated that the 90 days case fatality rate from HIV-associated CM in East Asia, Oceania, Western Europe and The US is 9%, compared with 55% in other parts of Asia and South America and 70% in sub-Saharan Africa. Now a day's Cryptococcosis disease occurs after 2.8-8% of solid organ transplants, and this disease is third most common fungal infections, after *Candida* and *Aspergillus*. In a review of US data from 1996 to 2010, kidney-transplant recipients. Symptoms may emerge sooner after lung or liver transplants, perhaps because the required level of post-operative immunosuppression is higher.

Amphotericin B (AmB), the drug with the greatest early fungal activity, which is administered intravenously for 14 days during induction therapy whenever possible. Its activity is concentration dependent. Flucytosine should accompany AmB during induction therapy at an intravenous or oral dose. As flucytosine remains unlicensed in most Asian and African countries and hence that the alternative agents have been considered for combination with AmB. The obvious contender is fluconazole [3].

DISEASE SIGN AND SYMPTOM

Many of the symptoms of CM are similar to those seen in other disease. These include fever, fatigue; stiff neck, body aches headaches (often severe), nausea/vomiting, and skin lesions. Other symptoms include confusion, muddles thinking, vision problems and possibly seizures, cough, dyspnea, *Molluscum contagiosum*.

People diagnosed with CM often have symptoms of infection outside the brain. This includes coughing and shortness of breath from infection in the lungs and skin lesions that can look like another infection called *M. contagiosum*. It is always advisable for HIV-positive people to report any symptoms now matter how mild, to their health-care provider [1,2].

There are two-ways to diagnose CM. The first involves looking for the fungus in the bloodstream. This is nothing more than a simple broad test. The second, most common way to diagnose CM involves the liquid - The cerebrospinal fluid (CSF) - that surrounds the brain and spine. To collect this fluid by a doctor or a technician must perform a lumber puncture also called a spinal tap. Once a small amount of CSF has been removed from the spine, a laboratory can look for CM in the

fluid. A spinal tap also done to check the amount of pressure in the brain. Because CM can cause the brain to swell, the pressure of the CSF can increase. Knowing the CSF pressure can help to determine how severe the disease is.

In some cases, the infected person may experience a stiff neck and fever.

If left untreated, CM may lead to more serious symptoms such as:

- Brain damage
- Coma
- Hearing loss
- Hydrocephalus (water on the brain).

Drug of choice both conventional first line and second line

Both the Infectious Disease Society of America and 2011 WHO rapid advice guidelines recommend AmB and flucytosine as the first line induction treatment for patients with CM, with alternative regimens tailored to individual clinical settings. For settings in which flucytosine is unavailable, second line induction treatment consists of AmB and high dose (800-1200 mg/day) fluconazole. Where AmB is unavailable or cannot be safely given and monitored, high dose fluconazole and flucytosine is recommended. The initial 2 weeks induction treatment is followed by consolidation and maintenance phases of treatment with fluconazole [4].

Challenging perspectives

AmB has a broad antifungal range, and only a few reports of resistance have been documented. However, it has some side-effects such as anemia, hypokalemia, hypomagnesaemia and nephrotoxicity. By intravenous administration, monitoring of blood count and renal function, and perception of unmanageable toxic effects frequently prevent the use of AmB in poorly resourced and understaffed hospital settings. Increasing doses of amphotericin to 1 mg/kg/day as compared with 0.7 mg/kg/day are more fungicidal. In the absence of underlining renal disease or clinically significant anemia, higher doses may be preferred, although no differences have been observed in mortality between these two doses [2,4].

Flucytosine is a potential antitumor agent. The use of originally restricted because of the drug's toxic effects at high doses (150 mg/kg/day). Flucytosine exerts its antifungal activity through rapid conversion into 5-fluorouracil and is available in intravenous and oral formulations, marketed as ancotil 2.5 g/250 ml solution for infusion and ancobon 500 capsules. Although flucytosine is a nucleotide analogue of simple chemical structure that has been off-patent for many years, there seems to be market failure because of insufficient demand and supply [2,4].

Fluconazole has excellent bioavailability and CSF penetration and few adverse effects. The drug is available in intravenous formulation and is commonly given orally to treat CM. Clinical and mycological outcomes in trials of low-dose fluconazole monotherapy (200-400 mg/day) as induction treatment have been disappointing, with high mortality and prolonged time to CSF sterilization. It also has less availability to fulfill 1200 mg/kg/day [2,4].

Novel therapeutic approach

To improve the therapeutic efficacy and to reduce the toxicity of AMPH-B new drug delivery systems have been used. New drug delivery systems such as liposomal formulations, lipid complexes, and colloidal dispersions, have been introduced, and research studies and clinical trials are in progress. Because of better tolerability, lipid formulations are used more frequently in many high-income countries due to lower toxicity [5]. AmB is the most rapidly acting fungicidal agent against *C. neoformans*. On the basis of evidence from clinical trials, treatment guidelines for CM recommended 2 weeks of AmB based treatment as the first line treatment. Although prohibitively expensive for in low income and middle income countries patients, liposomal formulations of AmB allow the delivery of high doses of amphotericin and seem to be at least as effective and less nephrotoxicity than conventional AmB [6]. Whenever AmB deoxalate is administered, supplemental potassium 40-60 mEq/day and magnesium 8-16 mEq/day are required, with increasing amounts in the 2nd week of amphotericin. Without routine supplementation, the majority of persons will develop severe hypokalemia by the 2nd week of amphotericin, which can be life-threatening [2-4].

***In vivo* test**

The guidelines for animal experimentation of the Nagasaki University Laboratory Animal Centre for Biomedical Research had been followed. Under general anesthesia 6-weeks old BALB/c male mice purchased had been inoculated intratracheally through gavage with 50 µl. of cell suspension containing 10^5 cells of *C. neoformans* in normal saline. The final concentrations of the AMPH-B formulations had been adjusted to 0.8 or 2.0 mg/kg of body weight in 5% dextrose had been injected via the lateral tail vein once daily for 5 days beginning 2 hrs after the inoculation (day 0). The mice had been observed for survival daily for 60 days. The animals were sacrificed 7 days after inoculation and the lungs had been removed, suspended in sterile saline, and homogenized. A volume of 50 µl of 10-fold serially diluted suspension had been inoculated on Saboured dextrose agar and incubated at 35°C for 48 hrs. And the colonies had been counted [7].

Pharmacodynamic effect and pharmacokinetic effect

Fungizone, AmBisome and NS-718 were used in a study. Ns-718 is a lipid nanospehere encapsulated AMPH-B, 1 g each of soyabean oil and 2 g of maltose in each vial was taken. Blood and lung of mice inoculated with *C. neoformans* were collected at time points of 10 minutes and 2, 4, 6, 12 and 24 hrs after intravenous injections of fungizone, ambisome, or NS-718 at 0.8 and 2.0 mg/kg doses. While the mice were under anesthesia whole blood was collected from axillary vessel. A thoracotomy was performed. The lung were perfused with normal saline and then removed surgically. Lung was homogenized with methanol containing 1-amino 4 nitropthalene. The concentration of AMPH-B was determined by HPLC according to the method of Granich *et al*. with some modifications. By measurement of minimum inhibitory concentrations (MIC) the activity of antifungal drugs is calculated. Here the concentration of AMPH-B in serum had been seen highest (17.4 µg/g) after administration of 0.8 mg of NS 718/kg. At 2 hrs the concentration NS-718 administration had been lower (1.04 µg/g) than that of AmBisome but higher than fungi zone administration. After administration of 2.0 mg of NS-718/kg, the concentration of AMPH-B in serum at 10 minutes had been higher than that of AmBisome administration. The concentration of AMPH-B in lung at 10 minutes was the same after administration of 0.8 mg of NS-718 or AmBisome [7].

Statistical analysis

Each experiment had repeated at least twice to ascertain the reproducibility. Data had expressed as means standard deviations. Tests for differences in survival distributions had been based on a generalized Wilcoxon test from survival rates calculated by the Kaplan–Meier method. The mean numbers of colony forming units per gram of lung tissue from the mycological study had been compared by Scheffe's multiple-comparison test. A $p<0.05$ had considered statistically significant. The MIC of antifungal agents had been determined by the microdilution method modified from the macro dilution method of the National Committee for Clinical Laboratory Standards. In case of 50% and 90% MICs of AMPH-B had been shown as 1 and 2, in case of Fungizone MICs are 0.5 and 0.5, in case of AmBisome MICs are 0.5 and 1 and for NS 718 MICs had been shown 0.0625 and 0.125 [7].

Is it really curable?

No. Because this disease has high mortality rate, and AmB has serious toxicity. In a recent study, NS-718 had been shown the most effective lipid nanospehere encapsulated formulation of AMPH-B against Cryptococcosis [6]. In a multicenter study, AmB lipid complex had been found to have apparently better clinical and microbiological activity against CM in patients with AIDS and had been significantly better tolerated than AmB but that was not free of toxicities. In another cases,

amphotericin and its lipid formulations have a high cost which is not comfort to the patient [8,9].

DISCUSSION

From a study of we found that, NS-718, a lipid nanospehere AmB had been showed that a potential effect at a dose of 0.8 mg/day with respect to AmBisome, and at a highest dose of 2.0 mg it has also shown MIC in comparison with fungizone eventually it has better therapeutic property than the other comparator drugs [10]. NS-718 had been showed powerful activity than AmBisome, because the release of AMPH-B from AmBisome was slight and slower [11,12].

CONCLUSION

From ASM journal we have seen that lipid nanospehere encapsulated AmB has high effectiveness and potency than AmB. But it has toxicity. AmB has high cost, toxic effects and insufficiently coordinated distribution, flucytosine through challenges to maintenance of local stocks, is it through sustainability of donations or insufficient generic supplies and in case of lipid formulations of amphotericin also have some toxic effects. The results of the present studies are encouraging and further investigations for evaluation of NS-718 in treatment of comparative study profiles are needed to establish NS-718 as the most effective antifungal agent in human Cryptococcal infection. So we can conclude that we have to take further better action to cure this disease [7,13].

REFERENCES

1. Saag MS, Powderly WG, Cloud GA, Robinson P, Grieco MH, Sharkey PK, *et al.* Comparison of amphotericin B with fluconazole in the treatment of acute AIDS-associated *Cryptococcal meningitis*. The NIAID Mycoses Study Group and the AIDS Clinical Trials Group. N Engl J Med 1992;326(2):83-9.
2. Bicanic T, Harrison TS. *Cryptococcal meningitis*. Br Med Bull 2005;72:99-118.
3. Sloan DJ, Parris V. *Cryptococcal meningitis*: epidemiology and therapeutic options. Clin Epidemiol 2014;6:169-82.
4. Loyse A, Thangaraj H, Easterbrook P, Ford N, Roy M, Chiller T, *et al. Cryptococcal meningitis*: improving access to essential antifungal medicines in resource-poor countries. Lancet Infect Dis 2013;13(7):629-37.
5. George RJ, Jennings AL. Palliative medicine. Postgrad Med J 1993;69(812):429-49.
6. Hsin IC, Ming YC, Ming KY. Clinically-proven liposome based drug delivery: Formulation, characterization and therapeutic efficacy. Open Access Sci Rep 2012;3(1):2-8.
7. Hossain MA, Maesaki S, Kakeya H, Noda T, Yanagihara K, Sasaki E, *et al*. Efficacy of NS-718, a novel lipid nanosphere-encapsulated amphotericin B, against *Cryptococcus neoformans*. Antimicrob Agents Chemother 1998;42(7):1722-5.
8. Olson JA, Adler-Moore JP, Jensen GM, Schwartz J, Dignani MC, Proffitt RT. Comparison of the physicochemical, antifungal, and toxic properties of two liposomal amphotericin B products. Antimicrob Agents Chemother 2008;52(1):259-68.
9. Ostrosky-Zeichner L, Rex JH, Pappas PG, Hamill RJ, Larsen RA, Horowitz HW, *et al.* Antifungal susceptibility survey of 2,000 bloodstream *Candida* isolates in the United States. Antimicrob Agents Chemother 2003;47(10):3149-54.
10. Hata K, Kimura J, Miki H, Toyosawa T, Moriyama M, Katsu K. Efficacy of ER-30346, a novel oral triazole antifungal agent, in experimental models of Aspergillosis, Candidiasis, and Cryptococcosis. Antimicrob Agents Chemother 1996;40(10):2243-7.
11. Leite AG, Vidal JE, Bonasser Filho F, Nogueira RS, Oliveira AC. Cerebral infarction related to *Cryptococcal meningitis* in an HIV-infected patient: case report and literature review. Braz J Infect Dis 2004;8:175-9.
12. Daneshtalab M. Discovery of chlorogenic acid-based peptidomimetics as a novel class of antifungals. A success story in rational drug design. J Pharm Pharm Sci 2008;11:44s-55.
13. Joshua R, David RB. Prognosis and management of *Cryptococcal meningitis* in patients with human immunodeficiency virus infection. Neurobehav HIV Med 2012;4:45-61.