ประสิทธิผลและความปลอดภัยของการนำยาฟลูโคนาโซลมาใช้ร่วมกับยาแอมโฟเทริซิน บี ในการรักษาโรคเยื่อหุ้มสมองอักเสบจากการติดเชื้อราคริปโตค็อกคัสในผู้ป่วยเอดส์

นายชาญกิจ พุฒิเลอพงศ์

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EFFICACY AND SAFETY OF FLUCONAZOLE COMBINED WITH AMPHOTERICIN B FOR TREATMENT OF CRYPTOCOCCAL MENINGITIS IN PATIENTS WITH AIDS

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ชาญกิจ พุฒิเลอพงศ์ : ประสิทธิผลและความปลอดภัยของการนำยาฟลูโคนาโซลมาใช้ร่วมกับยาแอมโฟเทริซิน บี ในการรักษาโรคเยื่อหุ้มสมองอักเสบจากการติดเชื้อราคริปโตค็อกคัสในผู้ป่วยเอดส์ (EFFICACY AND SAFETY OF FLUCONAZOLE COMBINED WITH AMPHOTERICIN B FOR TREATMENT OF CRYPTOCOCCAL MENINGITIS IN PATIENTS WITH AIDS) อ.ที่ปรึกษา : รศ.ดร.ดวงจิต พนมวัน ณ อยุธยา, อ.ที่ปรึกษาร่วม : น.พ.สมสิทธิ์ ตันสุภสวัสดิกุล : 91 หน้า. ISBN 974-334-476-4.

โรคเยื่อหุ้มสมองจากการติดเชื้อราคริปโตค็อกคัส เป็นโรคติดเชื้อราฉวยโอกาสที่พบบ่อยและมีอันตรายถึงแก่ชีวิต การศึกษานี้มีวัตถุประสงค์ เพื่อศึกษาประสิทธิผลและความปลอดภัยของการใช้ยาแอมโฟเทริซิน บี ขนาดสูงใน 2 สัปดาห์แรก ร่วมกับยาฟลูโคนาโซล เปรียบเทียบกับยาแอมโฟเทริซิน บี อย่างเดียว โดยติดตามผลต่อเนื่องจนครบ 8 สัปดาห์ของการรักษา ในผู้ป่วยเอดส์จำนวน 72 ราย ในโรงพยาบาลบำราศนราดูร

ผู้ป่วยส่วนใหญ่เป็นเพศชาย (ร้อยละ 77.8) มีอายุอยู่ในช่วง 20-29 ปี ลักษณะอาการทางคลินิกที่พบมาก คือ อาการปวดศีรษะ (ร้อยละ 97.2) อาการคลื่นไส้-อาเจียน (ร้อยละ 84.7) และอาการไข้ (ร้อยละ 59.7) มีความดันน้ำไขสันหลังสูงกว่า 200 มม.น้ำ (ร้อยละ 91.7) และจำนวนเซลล์เม็ดเลือดขาวในน้ำไขสันหลังน้อยกว่า 20 เซลล์ (ร้อยละ 76.4) ใน 2 สัปดาห์แรก ผู้ป่วยจำนวน 38 ราย ถูกสุ่มให้ได้รับการรักษาด้วยยาแอมโฟเทริซิน บี 0.7 มก./กก./วัน ร่วมกับยาฟลูโคนาโซล 800 มก. ในวันแรก ต่อด้วย 400 มก./วัน ในวันถัดมา และผู้ป่วยอีก 34 ราย ได้รับการรักษาด้วยยาแอมโฟเทริซิน บี อย่างเดียวในขนาดที่เท่ากัน ต่อจากนั้นอีก 6 สัปดาห์ ผู้ป่วยทั้ง 2 กลุ่มจะได้รับการรักษาด้วยยาฟลูโคนาโซล 400 มก./วัน หรือ 200 มก./วัน ในผู้ป่วยที่มีผลเพาะเชื้อราเป็นลบในครั้งแรก ผลการรักษาที่ประสบความสำเร็จ จะพิจารณาจากอาการทางคลินิกที่ดีขึ้น ร่วมกับผลเพาะเชื้อราจากน้ำไขสันหลังที่เป็นลบติดต่อกัน 2 ครั้ง โดยห่างกันอย่างน้อย 1 สัปดาห์ ผลการรักษาในสัปดาห์ที่ 2 พบว่าผู้ป่วยส่วนใหญ่ในทั้ง 2 กลุ่ม มีอาการทางคลินิกที่ดีขึ้น แต่ยังมีผลเพาะเชื้อที่เป็นบวกอยู่ สำหรับผลเพาะเชื้อที่เป็นลบพบในผู้ป่วยจำนวน 9 ราย (ร้อยละ 23.7) ที่รักษาด้วยยาแอมโฟเทริซิน บี ร่วมกับยาฟลูโคนาโซล และ 11 ราย (ร้อยละ 32.4) ที่รักษาด้วยยาแอมโฟเทริซิน บี อย่างเดียว ซึ่งผลดังกล่าวไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ (P=0.37) เมื่อพิจารณาผลการรักษาในสัปดาห์ที่ 8 พบว่า มีผู้ป่วยจำนวน 24 ราย (ร้อยละ 63.2) ที่รักษาด้วยยาแอมโฟเทริซิน บี ร่วมกับยาฟลูโคนาโซล และ 22 ราย (ร้อยละ 64.7) ที่รักษาด้วยยาแอมโฟเทริซิน บี อย่างเดียว มีผลการรักษาที่ประสบความสำเร็จ และไม่พบว่ามีความแตกต่างอย่างมีนัยสำคัญทางสถิติ (P=0.47) สำหรับค่ามัธยฐานของระยะเวลาที่ผลเพาะเชื้อเปลี่ยนเป็นลบครั้งแรกของผู้ป่วยในแต่ละกลุ่ม อยู่ที่สัปดาห์ที่ 4 ของการรักษา ส่วนอัตราการตายในช่วง 2 สัปดาห์แรก พบเป็นร้อยละ 13.2 ในกลุ่มที่รักษาด้วยยาแอมโฟเทริซิน บี ร่วมกับยาฟลูโคนาโซล และ ร้อยละ 14.7 ในกลุ่มที่รักษาด้วยยาแอมโฟเทริซิน บี อย่างเดียว โดยไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ (P=1.00) ในระหว่างการรักษาไม่มีผู้ป่วยรายใดที่ต้องหยุดยา เนื่องจากอาการไม่พึงประสงค์จากการใช้ยา ผลการศึกษานี้ชี้ให้เห็นว่า การรักษาโรคเยื่อหุ้มสมองจากการติดเชื้อราคริปโตค็อกคัสในผู้ป่วยเอดส์ โดยการใช้ยาฟลูโคนาโซลร่วมกับยาแอมโฟเทริซิน บี ในช่วงสองสัปดาห์แรก ไม่ได้เพิ่มทั้งประสิทธิผลของการรักษาในด้านการทำให้น้ำไขสันหลังปราศจากเชื้อหรืออาการไม่พึงประสงค์จากการใช้ยา เมื่อเปรียบเทียบกับการใช้ยาแอมโฟเทริซิน บี เพียงอย่างเดียว แบบแผนการรักษาด้วยยาแอมโฟเทริซิน บี อย่างเดียวในขนาดสูง ใน 2 สัปดาห์แรก ให้ผลการรักษาที่ประสบความสำเร็จร้อยละ 64.7 ซึ่งสูงกว่าแบบแผนการรักษาที่รายงานไว้ในการศึกษาอื่นๆ ซึ่งพบความสำเร็จประมาณร้อยละ 35-50 ดังนั้นแบบแผนการรักษานี้ จึงแนะนำให้ใช้ต่อไปในอนาคต

ภาควิชา เภสัชกรรม ลายมือชื่อนิสิต .....

สาขาวิชา เภสัชกรรมโรงพยาบาลและคลินิก ลายมือชื่ออาจารย์ที่ปรึกษา .....

ปีการศึกษา 2542 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

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CHANKIG PUTTILERPONG : EFFICACY AND SAFETY OF FLUCONAZOLE COMBINED WITH AMPHOTERICIN B FOR TREATMENT OF CRYPTOCOCCAL MENINGITIS IN PATIENTS WITH AIDS. THESIS ADVISOR : ASSOC. PROF. DUANGCHIT PANOMVANA NA AYUDHYA, Ph.D. THESIS CO-ADVISOR : SOMSIT TANSUPHASWADIKUL, M.D. 91 PP. ISBN 974-334-476-4.

Cryptococcal meningitis is the most common life-threatening opportunistic fungal infection in patients with AIDS. The purpose of this study were to compare the efficacy and safety of giving high dose of amphotericin B for the first two weeks of therapy with or without fluconazole. The patients were observed for the total of eight weeks. The study was carried out in 72 cryptococcal meningitis patients with AIDS, at Bamrasnaradura Hospital.

Most of the patients were male (77.8%) ranging in aged from 20 to 29 years. The most common clinical symptoms were headache (97.2%), nausea or vomiting (84.7%) and fever (59.7%). Cerebrospinal fluid (CSF) pressure was higher than 200 mmH_2O (91.7%) and white blood cells were less than 20 cells/mm^3 (76.4%). For the first 2 weeks, 38 patients were randomly assigned to receive amphotericin B (0.7 mg/kg/d) plus fluconazole (800 mg loading dose on the first day and 400 mg daily on the later day, AMB+FLU) and 34 patients to receive amphotericin B alone (AMB) in the same dose for two weeks. The following six weeks both groups were treated with fluconazole (400 mg daily or 200 mg daily once the patients was found to have a first negative CSF culture). Treatment outcome was considered to be successful if the patient showed improvement in clinical signs and symptoms together with two consecutive negative CSF cultures at least one week apart. Outcomes of the patients at two weeks showed that majority of the patients in both groups had clinical outcome improvement but the mycological outcome was still persistence. The negative CSF cultures were found in 9 patients (23.7%) who received AMB+FLU and 11 patients (32.4%) who received AMB which was not statistically significant difference ($P = 0.37$). At eight weeks of therapy, the successful outcomes were found in 24 patients (63.2%) who received AMB+FLU and 22 patients (64.7%) who received AMB ($P = 0.47$). The median time to the first negative CSF culture was 4 weeks in each group. The mortality rate in the first two weeks was 13.2% in the AMB +FLU group and 14.7% in the AMB group which were not statistically significant ($P = 1.00$). None of the patients required discontinuance of the drugs during therapy due to adverse drug reactions. These results suggest that treatment of CM in patients with AIDS by the using fluconazole combined with amphotericin B in the first two weeks did not increase either the efficacy in term of sterilization of CSF culture or the adverse drug reactions when compared with the results obtained from the usage of amphotericin B alone. The regimen using high dose of amphotericin B alone for two weeks in this study resulted in the success rate of 64.7% which was higher than the success rate of 35-50% reported by other studies. This regimen is therefore recommended for future therapy.

ภาควิชา เภสัชกรรม ลายมือชื่อนิสิต .....

สาขาวิชา เภสัชกรรมโรงพยาบาลและคลินิก ลายมือชื่ออาจารย์ที่ปรึกษา .....

ปีการศึกษา 2542 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

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ABBREVIATIONS

ACTG	=	AIDS Clinical Trial Group
AIDS	=	Acquired immnunodeficiency syndrome
ALT	=	Alanine aminotransferase
AMB	=	Amphotericin B
AST	=	Aspartate aminotransferase
AUCs	=	Areas under the concentration-time curves
BUN	=	Blood urea nitrogen
CM	=	Cryptococcal meningitis
CRAG	=	Cryptococcal antigen
CSF	=	Cerebrospinal fluid
CT	=	Computed tomography
5-FC	=	Flucytosine
FLU	=	Fluconazole
HIV	=	Human immunodeficiency virus
ICP	=	Intracranial pressure
MRI	=	Magnetic resonance imaging
MSG	=	Mycoses Study Group
PCP	=	*Pneumocystis carinii* pneumonia
ULN	=	Upper limit of normal

CHAPTER I

INTRODUCTION

Cryptococcal meningitis (CM) is the most common life-threatening opportunistic fungal disease in patients with acquired immunodeficiency syndrome (AIDS). The fatality rate during initial therapy is 10-25% and the 12-month overall survival rate is 40-60%. Prior to the onset of the AIDS epidemic in the early 1980, neoplastic disease, organ transplantation, and immunosuppressive therapy were the most frequently recognized conditions or factors predisposing to this disease. Since 1980, AIDS has become the most frequent predisposing condition. Studies indicated that 5-10% of human immunodeficiency virus (HIV)-infected patients in Western countries and up to 30% of those in sub-Saharan Africa will develop cryptococcal meningitis.[1-3] According to the report from Division of Epidemiology, Ministry of Public Health of Thailand during September 1984 to January 2000, there were 22,937 Thai patients with cryptococcosis (16.9%) which was the third most common opportunistic infection in AIDS patients after *Mycobacterium tuberculosis*, Pulmonary or extrapulmonary and *Pneumocystis carinii* pneumonia (PCP).[4]

The treatment goal of cryptococcal meningitis for patients with AIDS differ from those patients who do not have AIDS. In patients with AIDS, complete cure is very unlikely but if untreated, cryptococcal meningitis is uniformly fatal in such patients. The treatment goals are control of infection, decrease in early mortality, prevention of relapse, and maintenance of the patient's quality of life.[2] Retrospective studies have reported relapse rate of 50-60% and shorter life expectancy for patients who did not receive chronic suppressive or maintenance therapy after termination primary treatment or initial therapy.[1-3] Consequently, all patients with AIDS associated cryptococcal meningitis require life long maintenance therapy to prevent relapse after completion of successful primary therapy.

Standard primary therapy for CM in AIDS patients consist of amphotericin B (AMB) either alone or combined with flucytosine (5-FC).[3] The optimal dosage of AMB and the optimal duration of its administration remain controversial, as do the relation efficacies of AMB alone and the combination of AMB with flucytosine. In many comparative studies, AMB plus flucytosine were shown to be superior to AMB alone both in overall response and in time to sterilization of cerebrospinal fluid (CSF).[1-2,5-11] However, the major limitation of prolonged use of the combination of AMB and flucytosine in patients with AIDS is their toxic effects. AMB is associated with significant nephrotoxicity, and flucytosine is associated with

myelosuppression and gastrointestinal toxicity. Thus, alternative antifungal agents treatment with less toxicity were studied, especially oral triazole drugs. The triazole antifungal agents, fluconazole and itraconazole have contributed greatly to our ability to treat cryptococcal meningitis in patients with AIDS. The response rates ranged from 50-60% were respected in noncomparative studies in patients who had received no prior therapy.[2,12] In many prospective studies, fluconazole alone (200-800 mg/day) had the response rate ranged from 35-75% and higher response rates were found in the groups of patient who received fluconazole with doses higher than 400 mg/day.[5-7,12-16] Recently, one randomized study of AMB combined with itraconazole compared with AMB alone in patients with AIDS associated CM showed that therapeutic success, defines as CSF culture negativity within 8 weeks, found appears to be beneficial with addition of itraconazole to AMB in treatment of CM.[8] In the treatment of CM, fluconazole appears to be better tolerated than AMB. It can be given orally or intravenously, has a long half-life and excellent penetration in CSF with approaching 80% of those attained in serum. The drug is well tolerated with infrequently seen adverse effect. Therefore, it is reasonable to hope that the addition of fluconazole combined with AMB could show higher clinical and mycological response those already exist regimens. However, despite all these facts, this issue has never been investigated.

Therefore, we designed a randomized clinical trial to compare the efficacy and safety of AMB with or without fluconazole in the treatment of cryptococcal meningitis in patients with AIDS.

Objectives

1. To compare the efficacy and safety of amphotericin B with or without fluconazole within 2 weeks as induction therapy for cryptococcal meningitis in patients with AIDS

2. To compare the efficacy and safety of amphotericin B with or without fluconazole in the next additional 6 weeks as consolidation therapy for cryptococcal meningitis in patients with AIDS

The significance of the study

1. This study will provide the information on the efficacy and safety of amphotericin B with or without fluconazole which can be used as the data for selecting the appropriate dosage regimen to maximize the efficacy while minimizing the side effect.

2. This study will provide the information for pharmaceutical care in AIDS patients associated with cryptococcal meningitis.

CHAPTER II

REVIEW OF LITERATURE

1. Cryptococcal meningitis

Cryptococcal meningitis (CM) is the most common life-threatening opportunistic fungal infection in patients with AIDS, which caused by *Cryptococcus neoformans*. The organism is inhaled and dissemination occurs with predilection for the central nervous system. Disseminated infection may involve the bones, joints, and skin. The large polysaccharide capsules surrounding this organism is responsible for inhibition of phagocytosis, altered cellular immunity, immune paralysis, and tolerance. Prior to the onset of the AIDS epidemic in the early 1980s, neoplastic disease, organ transplantation, and immunosuppressive therapy were the most frequent predisposing condition.The incidence of cryptococcal meningitis has increased considerably and is considered the principal life-threatening opportunistic fungal infection. Many studies indicated that 5-10% of HIV-infected patients in Western countries and up to 30% of those in sub-Saharan Africa developed cryptococcal meningitis. [2-3]

A. Etiology [17-18]

Cryptococcus neoformans is an encapsulated yeast that measures 4-6 μm in diameter surrounded by a capsule that is 1-30 μm. A perfect form or sexual stage can be produced *in vitro*; however, it has not been found in nature. Thus, the asexual, yeast form is considered to be the primary infections agent. Most isolates grow readily on bacterial or fungal media within a week after inoculation. Occasionally, 3-4 weeks is required for growth; therefore, all plates should be held for 1 month before declaring the culture negative. The organism is differentiated from nonpathogenic cryptococcal species by its ability to grow at 37°C and to convert phenolic compounds to melanin, producing dark colonies when incubated in niger seed extract or similar media. Its ability to produce urease helps in the rapid identification of the organism.

Glucuronoxylomannan is the predominant capsular polysaccharide and is the principal antigenic domain. There are four serotypes as determined by capsular antigens. Serotypes A and D are classified as *C. neoformans* var. *neoformans* and serotypes B and C are classified as *C. neoformans* var. *gattii*. Human infection caused by cryptococci other than *C. neoformans* has been reported, especially *Cryptococcus albidus* and *Cryptococcus laurenti*, but these are of doubtful clinical significance.

Cryptococcus neoformans var. *neoformans* exists, most likely, in common grass or cereal that birds, and pigeons in particular, eat. The organism does not cause infection in birds, but the droppings from pigeons and soil contaminated with avian droppings are important sources of infection in humans. These serotypes B and C are isolated from humans in Southern California, and from tropical and subtropical areas of the world. Interestingly, only serotypes A and D are usually isolated from AIDS patients, the most prevalent is serotype A.

B. Epidemiology [4,17-18]

Cryptococcus neoformans var. *neoformans* is the most frequent cause of infection worldwide, accounting for close to 100% of the clinical isolates from Europe and Japan and more than 85% of the isolates in the United States (excluding Southern California), Canada, and Argentina. *Cryptococcus neoformans* var. *gattii* is more frequent in tropical and subtropical regions of Australia, Brazil, Southeast Asia, Central Africa and Southern California and in the pre-acquired immunodeficiency syndrome era, constituted 35-100% of all clinical isolates. With the advent of the AIDS epidemic the percentage of cases caused by var. *gattii* has decreased significantly, due in large part to its tendency to infect immunocompetent hosts. Most cases in AIDS patients are caused by var. *neoformans*. Cryptococcosis occurs in 5-30% of patients with AIDS. According to the report from Division of Epidemiology, Ministry of Public Health of Thailand during September 1984 to January 2000, there were 22,937 Thai patients with cryptococcosis (16.9%) which was the third most common opportunistic infection in AIDS patients.

C. Pathogenesis [17-19]

Uncapsulated or partially encapsulated yeasts released to the environment are inhaled and deposited in small airways. In the immunocompetent host, a granulomatous response producing a primary complex, similar but less exuberant than the one found in primary tuberculosis, usually controls the infection. Patients with disease limited to be lung often have no major defects of cell-mediated immunity. If the immune response is defective, proliferation and extrapulmonary dissemination follow. When *C. neoformans* escapes from the lungs, the major secondary site of infection is the meninges. It is not clear why cryptococci are neurotropic, but cryptococcal meningitis is eventually fatal if it is not treated. *C. neoformans* elicits a chronic inflammatory response in infected hosts. In the meninges, this may involve the aqueduct of Sylvius, producing obstructive hydrocephalus.

There may also be vasculitis, with subsequent focal ischemic damage to the brain or cranial nerves. There may be diffuse inflammation of the meninges, which impairs reabsorption of cerebrospinal fluid and causes communicating hydrocephalus. Extracerebral foci of infection may also include the skin, bones, and other soft tissues.

Cryptococcal neoformans produces no toxins and evokes very little inflammatory response. Its main virulence factor is the capsular polysaccharide. A capsular mutants have substantially reduced virulence. Regulation of capsule production is an adaptive process. The uncapsulated state promotes growth, mating, and penetration into the small airways. In the host, the larger capsule provides resistance to phagocytosis. Severely immunodeficient patients may exert little selective pressure for capsule production. Some AIDS patients have variants with small capsules that later produce large capsules when inoculated into animals. The carbon dioxide tension found in mammalian tissues may also be a stimulus for capsule production. The polysaccharide has also been shown to induce T-suppressor cell activity in experimental animals, which may depress T-cell dependent functions such as induction of macrophage response to yeast cells. Antibody plays a role in the phagocytosis and killing of cryptococci, but its clinical significance is uncertain. Cellular immunity is essential for control of the infection, which is why persons with HIV infection or lymphoreticular malignancies or those using corticosteroids are particularly prone to develop disseminted disease.

D. Clinical manifestations [2-3,17-18,20-21]

The course of cryptococcal meningitis is usually subacute, with a median time from onset of symptoms to diagnosis of 30 days. Most patients about 75-90% present with features of a subacute meningitis or meningoencephalitis with fever and headache are generally symptomatic for 2-4 weeks prior to presentation. More specific symptoms of meningeal involvement such as stiff neck, photophobia, nausea, and vomiting are present in only 20-40% of patients. A minority of patients about 30% also have symptoms compatible with encephalopathy such as lethargy, altered mental status, personality changes, and memory loss. Papilledema is found in less than 10% of patients. Cryptococcomas are rare in patients with AIDS. Although abnormalities on brain imaging (computed tomography or magnetic resonance imaging) are seen in up to 20% of patients, focal neurological signs or seizures are unusual and occur in only about 10% of patients.

The occurrence of concomitant extraneural disease in patients with cryptococcal meningitis ranges between 20% and 60% and may often lead to the diagnosis of meningitis. The most frequent sites of extraneural culture isolation is the blood. Even though the lungs are the portal of entry, symptoms of pulmonary disease occur in only 20-30% of patients. Cutaneous involvement is common, and although several types of skin lesions have been described. The most common form is that resembling molluscum contagiosum. Other sites of involvement include the oral cavity, eyes, bone marrow, liver, spleens, lymph nodes, pericardium, mediastinum, and genitourinary tract, which is believed to provide a reservoir for *C. neoformans*.

Pulmonary cryptococcosis

Symptoms of pulmonary cryptococcosis may be the initial manifestation of disease. Chest radiography typically shows bilateral alveolar or interstitial pneumonitis, although focal or nodular patterns, pleural effusions, and lymphadenopathy have also been described. The course of pulmonary cryptococcosis is also variable. In patients with normal hosts defenses, spontaneous regression is the rule, in contrast, pulmonary cryptococcal disease in immunocompromised hosts tends to be progressive and severe. The symptoms of primary infection include cough, scanty mucoid sputum owing to capsular polysaccharides, and infrequent hemoptysis. There may be a low-grade fever, pleuritic pain, and weight loss, but these symptoms are rarely predominant. A specific diagnosis of cryptococcal pulmonary infection may be established by cultures of sputum, bronchoscopic aspirate, bronchoscopic biopsy, or by detection of soluble antigen by the latex agglutination test. Because *C. neoformans* is normally absent from sputum, isolation of this organism is of diagnostic significance.

Central nervous system cryptococcosis

The high propensity of *C. neoformans* for the central nervous system explains why this form is the most frequently diagnosed. Usually, three clinical types are observed: meningitis, meningoencephalitis, and cerebral granuloma (cryptococcoma). Meningitis, usually subacute or chronic in nature, is the most common manifestation of central nervous system (CNS) disease caused by *C. neoformans*. Cryptococcomas are seen in about 5-20% of patients, most typically in those with meningitis, cryptococcomas appear to be less common in patients with AIDS than in other patient groups. Complications of CNS cryptococcosis include hydrocephalus, visual disturbances including blindness, hearing loss, other cranial nerve palsies, ataxia, seizures, and demantia. The mortality rate ranges from 15-30%, and most deaths occur within the first several weeks of illness.

Cutaneous cryptococcosis

Primary cutaneous cryptococcosis is usually post-traumatic, localized at the nose or finger, and benign in appearance. Secondary cutaneous cryptococcosis is a severe manifestation that appears in about 10-15% of immunosuppressed patients with disseminated cryptococcosis. The face, scalp, neck, trunk, and extremities are the common localizations of the papular, nodular acniform lesions which mimic molluscum contagiosum in both non-AIDS and AIDS patients. These lesions may become necrotic ulcerations.

Osseous cryptococcosis

Bones and joints are involved in 5-10% of disseminated cases of cryptococcosis. Every bone or articulation may be attacked, with a propensity for the long bones, cranial bones, and vertebrae. Osteomyelitis is more common than septic arthritis.

Visceral cryptococcosis

In disseminated cryptococcosis, any organ or tissue may be infected, including heart, testis, prostate gland, eyes, kidneys, adrenals, liver, spleen, and lymph nodes. The prostate gland appears to be an important reservoir of infection, which may serve as a source of relapse after completion of appearently successful primary therapy.

E. Diagnosis [17-18,20-23]

Cryptococcal meningitis should be suspected in the HIV-infected patients who presents with fever and/or headache, particularly with a CD_4^+ count below $100/mm^3$. In patients who present with extraneural cryptococcosis, it is mandatory to exclude meningeal involvement. The conventional diagnosis approach is to obtain a brain-imaging scan to exclude mass effect and other CNS process. One noncommunicating hydrocephalus and mass effects are ruled out, a lumbar puncture should be performed.

Brain-imaging studies

There are no specific radiological findings of cryptococcal meningitis. The computed tomography (CT) of the head is normal or shows cerebral atrophy presumably due to HIV-infection in 75-90% of patients. Nonenhancing and contrast-enhancing lesions, presenting as either nodular or ringlike patterns, are described in 8-15% of patients. Hydrocephalus and diffuse cerebral edema are less common.

Cerebrospinal Fluid Analysis

Cerebrospinal fluid (CSF) indicies are nonspecific and may be appear normal in cryptococcal meningitis:

- Opening pressure (OP) is usually elevated, exceeding 200 mmH_2O in two-third of patients. Patients with markedly elevated opening pressure should undergo CT or magnetic resonance imaging (MRI) to rule out communicating hydrocephalus. Although the exact mechanism of elevated intracranial pressure (ICP) is uncertain, the large number of yeast cells and/or free polysaccharide antigen have been postulated to occlude the channels and values of the subarachnoid villi and the lymphatic pathways, thereby interfering with CSF reabsorption. The lack of ventricle dilation is probably due to the coexistence of cerebral edema and diffuse parenchymal cryptococcal infiltration. Measurement of the opening pressure is essential for prognostic evaluation as well as clinical management.

- Glucose is depressed in only one-fourth of patients.
- Protein is elevated in about one-half of paients, but rarely exceeds 150 mg/dl
- Cell count typically shows mild lymphatic pleocytosis. Most patients have < 20 white blood cell/mm^3 of CSF. Only 20% of patients have > 20 white blood cell/ mm^3, and exceeding 200 white blood cell/mm^3 are rare.
- India ink staining is about 80% sensitive for detecting cryptococcosis CSF. Specificity is near 100%. However, the presence of cryptococcus in the CSF may persist during the first 2 month of treatment and does not indicate treatment failure during this period.

Cryptococcal antigen test

The cryptococcal antigen (CRAG) test is routinely performed on the CSF and serum. In patient with AIDS-related cryptococcal meningitis, the sensitivity of the CSF CRAG test is 91-100%, with titers that ranges from positive only when undiluted to 1:64,000. Serum CRAG has a sensitivity of 94-100% in HIV-infected patients with cryptococcal meningitis, with titers ranging from 1:1 to1:1,000,000. The use of serum CRAG for routine screening of HIV-positive patients is controversial. In addition to its limited value as screening test, a recently published review of the data from the large prospective studies on acute treatment and maintenance therapy for cryptococcal meningitis in patients with AIDS demonstrated no correlation between change in serum CRAG titers obtained during acute or suppressive therapy and outcome.

Cerebrospinal fluid culture

A positive cerobrospinal fluid culture is the definitive diagnostic test for cryptococcal meningitis and the main entry criteria in published studies. Being the gold standard, its sensitivity approaches, by definition, 100%.

F. Prognosis factors and outcome [2-3,17,20,22,24]

Cryptococcal meningitis is associated with significant morbidity and mortality, even if aggressive treated. The acute mortality during initial therapy due to AIDS-associated cryptococcal meningitis is 10-25%, and the 12-month survival rate among all patients is 40-60%. Many studies have evaluated factors associated with poor outcome in both AIDS and non-AIDS patients. The most important pretreatment factor associated with poor prognosis is abnormal mental status (lethargy, obtundation, or coma), at presentation. Other factors that appeared prediction of mortality during treatment included a cerebrospinal fluid cryptococcal antigen titer greater than 1:1,024, a low leukocyte count (< 20 cells/mm^3) in cerebrospinal fluid, age lesser than 35 years, positive extraneural cultures for *C. neoformans*, and hyponatremia.

G. Treatment

Anti-cryptococcal therapy should be started for patients with a clinical presentation, elevated serum CRAG, and CSF findings indicative of cryptococcal meningitis without awaiting culture results. The treatment goals of patients with AIDS and cryptococcosis differ from those patients with cryptococcosis who do not have AIDS. In patient with AIDS, complete cure is very unlikely, and the primary objectives are control of infection, decrease in early mortality, prevention of relapse, and maintenance of the patients quality of life.[2-3] Amphotericin B has long been considered as the mainstay of therapy for cryptococcosis. An attractive features of amphotericin B is the fungicidal action at high concentrations against some fungi, by contrast, this significant toxicity profiles of the drug compromises its usefulnesses. Flucytosine (5-FC) often is used in combination with amphotericin B to obtain the synergistic effect of this double-drug regimen. The azole group of antifungal drugs has enhanced the therapeutic as maintenance against *C. neoformans*, the newer triazoles, fluconazole and itraconazole, are the most effective agents in this class.

Initial therapy

All patients with cryptococcal meningitis require therapy. Before the discovery of amphotericin B, meningitis was always fatal. Since 1956, intravenous amphotericin B administered progressively daily or an alternate days at a dose of 1 mg/kg with a cumulative dose of 2-2.5 g over 2-3 months, has been shown to successfully cure 50-70% of cases, depending on the presence and severity of predisposing factors. The result of four prospective studies reported successful rate of 35-50% in patients who received 0.4-0.7 mg/kg/d of amphotericin B alone.[6-8,11] However, monotherapy with low dose amphotericin B in patients with AIDS and cryptococcal meningitis is associated with increased mortality. Intrathecal amphotericin B or subcutaneous reservoirs for intraventricular amphotericin B injections have been used in some cases when intravenous amphotericin B was not effective, but complications limit these procedures.

In 1970, the availability of flucytosine (5-FC) led to the studies on the combined chemotherapy to capitalize on drug synergy, in order to shorten the course of amphotericin B, to reduce the toxic dose of amphotericin B, and to avoid the development of resistant strains that have been observed with 5-FC monotherapy. The standard initial therapy for patients with AIDS-associated cryptococcal meningitis, amphotericin B alone or in combination with 5-FC, has been evaluated in numerous studies and resulted in overall rate of sterilization of cerebrospinal fluid of 40-100% with an acute mortality rate in 10-40%. Most of the higher rate of sterilization reported ranged from 60-100% were found in the patients who received combination of high dose of amphotericin B plus flucytosine with or without triazole antifungal agents for a long time more than 6 weeks.[5-11,25] The major limitation of prolonged use of combination of amphotericin B and 5-FC in patients with AIDS has been toxic effects. Amphotericin B is associated with significant infusion-associated morbidity and nephrotoxicity, and 5-FC is associated with myelosuppression and gastrointestinal toxicity.

The move away from amphotericin B and 5-FC, in the mid-1980s, the triazole antifungal agents such as fluconazole and itraconazole, which suggested, in doses of 200-400 mg/d, were effective in treating patients with AIDS and cryptococcal meningitis. The response rates about 50-60% were reported in noncomparative studies in patients who had received no prior therapy.[2,12] In addition, both fluconazole and itraconazole were used successfully in patients whose amphotericin B treatment failed or who developed dose-limiting toxic effects of amphotericin B. The triazoles also found favor because they are orally active and have minimal toxicity

particularly when compared with amphotericin B. The results from many studies on the use of fluconazole alone showed that the response rates about 35-75% that the higher response rates ranged from 50-75% were found in patients who received higher dose of fluconazole (> 400 mg/d).[5-7,12-16] In addition, the results of three randomized, prospective studies comparing triazole antifungal agents with amphotericin B as initial therapy for cryptococcal meningitis, the triazole antifungal agents were associated with clinical and mycological success rates about 50%.[5,7,25] Two of the studies,[5,25] both small, found a significantly better outcome with AMB regimen. Larsen and colleagues[5] found that 6 of 6 patients who received amphotericin B (0.7 mg/kg/d) plus 5-FC (150 mg/kg/d) had successful outcomes compared with a response rate of 43% (6 of 14) among patients randomized to receive fluconazole (400 mg/d). De Gans et al.[25] found that cultures for 9 of 9 patients assigned to receive amphotericin B (0.3 mg/kg/d) plus 5-FC (150 mg/kg/d) were negative at 6 weeks compared with those for 5 of 12 patients randomized to receive itraconazole.

The study conducted by the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) and the AIDS Clinical Trial Group (ACTG),[7] randomized patients to receive either amphotericin B or fluconazole (200 mg/d). The mean dosage of amphotericin B used in the study was 0.45 mg/kg/d. Forty percent (25 of 63) of patients treated with amphotericin B responded (defined as two negative cultures of CSF by 10 weeks of treatment) compared with 34% (44 of 131) of patients receiving fluconazole. There was no statistical difference between the two treatment arms. Mortality rate was 14% of patients receiving amphotericn B as compared with 18% of patients receiving fluconazole. In addition, the CSF was sterilized more rapidly in those patients receiving amphotericin B. The median time to negative cultures was 42 days for amphotericin B–treated patients and 64 days for fluconazole–treated patients. Although, the differences between the two treatment groups were not significant. A more rapid clearance of CSF with amphotericin B was also noted in the study of Larsen et al.[5]

The low overall response rate to standard treatment in the MSG/ATCG study might be explained by the use of lower doses of amphotericin B or by the use of a stricter definition of successful treatment than used in previous studies. In addition, the population may have presented with more advanced disease, with 27% of the patients having altered mental status at baseline. These considerations have led many authorities to recommended higher dose amphotericin B (≥ 0.7 mg/kg/d) with or without 5-FC, as first-line treatment.

Further support for this approach to management of cryptococosis was conducted by Fausto de Lalla and colleagues;[1] all patients were treated with high dose of amphotericin B (1 mg/kg/d) for 14 days followed by maintenance therapy with fluconazole or itraconazole. Therapy was successful in 29 (93.5%) of all 31 patients and none died of cryptococcosis. This result indicated that an aggressive approach to the primary treatment of cryptococcosis in AIDS patients because the administration of a relatively high dose of amphotericin B for a relatively short period, is effective and well tolerated.

More recently, a double-blind multicenter trial was conducted by the National Institute of Allergy and Infectious Diseases Mycoses Study Group and the AIDS Clinical Trial Group in 1997.[11] The groups of investigators randomly assigned patients with a first episode of AIDS associated cryptococcosis to treatment with higher-dose amphotericin B (0.7 mg/kg/d) with or without 5-FC (100 mg/kg/d) for two weeks as induction therapy, followed by eight weeks of treatment with itraconazole (400 mg/d), or fluconazole (400 mg/d) as consolidation therapy. The results of this study reported that the use of higher-dose amphotericin B plus 5-FC was associated with an increased rate of CSF sterilization and decreased mortality at two weeks, as compared with regimen used in the previous studies. Although consolidation therapy with fluconazole is associated with a higher rate of CSF sterilization, itraconazole may be a suitable alternative for patients unable to take fluconazole. In addition, there is still an interest in finding effective alternatives to amphotericin B. Three currently investigational options included some forms of an alternative formulation of amphotericin B, higher doses of fluconazole, and novel combination therapies.

In a study by Alexander et al. and collaborators,[26] 28 patients were randomized to treat with either liposomal amphotericin B (Ambisome) 4 mg/kg daily or standard amphotericin B 0.7 mg/kg/d for 3 weeks. Ambisome therapy resulted in a significantly earlier CSF culture conversion than amphotericin B, had equal clinical efficacy and was significantly less nephrotoxic.

There were also preliminary data on the use of higher doses of fluconazole. Berry et al.[16] used fluconazole (800 mg/d) as salvage therapy for severe patients for whom previous therapy had failed and noted responses in four patients. Haubrich et al.[13] reported complete responses in five of six patients with cryptococcal meningitis who were treated with 800 mg/d of fluconazole. The median time to conversion of CSF cultures to negative was 21 days. Francesco et al.[14] used fluconazole (800-1,000 mg/d IV) for 3 weeks

and then orally to treat 14 patients with AIDS and cryptococcal meningitis. At 10 weeks the rate of clinical success was 54.5% and the median time to the first negative CSF culture was 33.5 days. Furthermore, high dose fluconazole might be an effective and well-tolerated therapeutic option for patients with AIDS and acute cryptococcal meningitis.

The use of fluconazole (400 mg/d) in combination with 5-FC (150 mg/kg/d) was evaluated in 32 patients by the California Collaborative Treatment Group[6] with clinical success in 20 of 32 (63%) and culture conversion was 23 days. 5-FC had to be stopped because of toxicity in 28% of patients. Harriet et al.[27] designed the study to compare the combination therapy with fluconazole (200 mg/d for 2 months) and 5-FC (150 mg/kg/d for the first 2 weeks) with fluconazole alone at the same dose for 2 month. This combination therapy prevented death within 2 weeks and significantly increased the survival rate among these patients (32%) at 6 months over that among patients receiving monotherapy (12%). These data indicate that treatment with fluconazole and short-term 5-FC is a cost-effective and safe regimen that improves the quality of life for patients with AIDS-associated cryptococcal meningitis.

Recent data from the randomized study compared the combination of amphotericin B with itraconazole and amphotericin B alone. Patients who received amphotericin B with itraconazole had therapeutic success rate (83%) higher than that as the patients who received amphotericin B alone. The addition of itraconazole to amphotericin B appears to be beneficial in cryptococcal meningitis. However, all of these data could not be concluded which was the best regimen to treatment for cryptococcal meningitis in patients with AIDS. Further investigation about the therapeutic regimen that gives the highest efficacy and the lowest toxicity is needed in such group of patients.

Management of increased intracranial pressure [8,20,27]

An additional therapeutic issue in acute cryptococcal meningitis is the management of raised intracranial pressure which can be present at diagnosis or can develop during therapy. Increased intracranial pressure (OP > 200 mmH_2O) occurs in more than 20% of AIDS patients with cryptococcal meningitis, usually in the form of communicating hydrocephalus without radiological evidence of enlarged ventricles or cerebral edema in most cases. Its role in morbidity and mortality among AIDS patients has not been systematically evaluated. Intracranial hypertension may be both life-threatening and vision-threatening. Possible therapeutic approaches to patients with symptomatic intracranial hypertension include the mechanical drainage,

the use of an intraventricular shunt, lumbar drain, a daily lumbar puncture (removing) 25-30 ml of CSF, and the use of acetazolamide to inhibit production of CSF in the choroid plexus. The use of corticosteroids in this setting is controversial and cannot be routinely recommended. Consequently, many investigators believe that routine measurement of intracranial pressure and management of raised intracranial pressure are critical components of therapy for such patients and can ameliorate the sequelae of elevated intracranial pressure.

Maintenance therapy

After successful initial therapy, 50-60% of AIDS patients with cryptococcal meningitis relapse if no suppressive therapy is orally administered within the first 6 months.[1-3] These relapses are caused by failure to completely eradicate *C. neoformans*, especially in sanctuary sites such as the urinary tract. Maintenance therapy is aimed at suppressive the multiplication of the residual organisms, which the debilitated immune system is unable to suppress.

Fluconazole (FLU) is the suppressive treatment of choice. A large placebo-controlled study by the California Treatment Group compared fluconazole with placebo in patients who had been treated for acute cryptococcal meningitis with amphotericin B. Fluconazole was superior to placebo, with 3% of fluconazole-treated patients and 37% of placebo-treated patients relapsing at any site. Fifteen percent of placebo recipients had recurrent cryptococcal meningitis, compared to no case of meningitis in the fluconazole group.[29] An MSG-ACTG study compared daily therapy with fluconazole (200 mg/d) to weekly amphotericin B (1 mg/kg) in 189 patients followed for a median of 286 days. Relapses occurred in 14 of 78 patients (18%) in the amphotericin B arm versus only 2 of 111 patients (2%) assigned to the fluconazole arm.[30] More recently, the National Institute of Allergy and Infectious Disease Mycoses Study Group compared the effectiveness of fluconazole versus itraconazole as maintenance therapy for AIDS-associated cryptoccocal meningitis. Relapse rates were 13 of 57 (23%) among itraconazole recipients and 2 of 51 (4%) among fluconazole recipients. Thus, fluconazole at dose of 200 mg/d remains the treatment of choice for maintenance therapy after initial therapy of cryptococcal meningitis in patients with AIDS.[31]

2. Drugs therapy

2.1 Amphotericin B [32-36]

Chemistry and stability

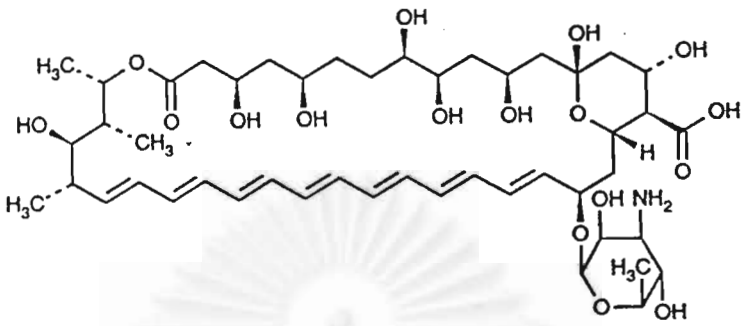
Amphotericin B, the first commercially available systemic antifungal drug, remains the cornerstone for therapy in critically ill patients with systemic fungal infections. It was first isolated in the 1950s from *Streptomyces nodosus*, an actinomycete cultured from the soil of the Orinoco Valley in Venezuela. It is a polyene macrolide that consists of seven conjugated double bonds, an internal ester, a free carboxyl group and a glycoside side chain with a primary amino group (Figure 1). Amphotericin B is amphoteric, forming soluble salts in both basic and acidic environments. It is not orally or intramuscularly absorbed, and is virtually insoluble in water. The intravenous infusion is commercially formulated as a deoxycholate micellar suspension consisting of amphotericin 50 mg and sodium deoxycholate 41 mg with sodium phosphate 20.2 mg as buffer.

Amphotericin B powder for injection should be protected from moisture and light and stored at 2-8°C. Amphotericin B powder for injection must be reconstituted only with sterile water for injection because precipitation may occur in solution containing is sodium chloride or bacteriostatic agents such as benzyl alcohol are used. Reconstituted preparation should be protected from light and stable for 24 hours at room temperature or 1 week when refrigerated at 2-8°C. For IV administration, amphotericin B must be diluted only with 5% dextrose in water having a pH greater than 4.2.

Mechanism of Actions

Amphotericin B is fungicidal depending on the concentration obtained in body fluid and the susceptibility of the fungus. It acts by binding irreversible to ergosterol, the principle sterol present in the cell membrane of sensitive fungi. This binding alters membrane permeability, causing leakage of sodium, potassium and hydrogen ions, eventually leading to cell death. It also binds at a lesser extent to other sterol, such as cholesterol, which is the component of the mammalian cell leading to cytotoxicity.

Figure 1: Chemical structure of amphotericin B



Microbiology

Amphotericin B deoxycholate is active *in vitro* against many species of fungi. *Histoplasma capsulatum*, *Coccidioides immitis*, *Candida* sp., *Blastomyces dermatitidis*, *Rhodotorula* sp., *Cryptococcus neoformans*, *Sporothrix schenckii*, *Mucor mucedo* and *Aspergillus fumigatus* are inhibited by concentrations ranging from 0.03-1 mcg/ml *in vitro*. It has no effect on bacteria, rickettsiae, and viruses.

Pharmacokinetics

Absorption

Amphotericin B is poorly absorbed following oral administration. Amphotericin B is extremely irritant when injected into muscle and this route should not used in human. Amphotericin B must be given parenterally to treat systemic fungal infections. Consequently, it needs to be given intravenously for the treatment of systemic fungal infections, but when given by this route it invariably causes side effects. These may be minimized by initially giving small doses which are then increased over 3-5 days. Amphotericin B is usually given as a slow intravenous infusion over 4-6 hours. Average peak plasma concentrations ranging from 0.5-2 mg/l are found in adults given repeated dose of approximately 0.5 mg/kg/day. These concentrations rapidly achieve a prolonged plateau phase of 0.2-0.5 mg/l.

Distribution

Following intravenous administration, amphotericin B is approximately 90-95% bound to serum proteins, primarily to lipoproteins, erythrocytes, and cholesterol in the plasma, and then redistributes from the blood into tissues. It is thought to follow a three compartment model of distribution (one central compartment and two peripheral compartment, one fast and one slow). The apparent volume of distribution of amphotericin B in man is about 4 L/kg. Amphotericin B in body fluids other than serum are generally quite low. Concentrations of amphotericin B in peritoneal, pericardial, aqueous humor, pleural, and synovial fluids are usually less than half those in the serum, while CSF concentrations range from undetectable to no more than 4% of serum concentrations.

Metabolism and eliminations

Metabolic pathways of amphotericin B are unknown and no metabolites have been identified. Amphotericin B follows a biphasic pattern of elimination from serum, with an initial half-life of 24-48 hours, follows by a long elimination half-life of up to 15 days, probably because of the extremely slow release of the drug from peripheral tissues. It has been eliminated that only about 3% of a total dose of amphotericin B is excreted in urine unchanged. The drug can be detected in blood up to 4 weeks and in urine up to 4-8 weeks after discontinuance of therapy. Excretion in the bile may represent an important route of elimination. Amphotericin B is not hemodialyzable.

Indications

Amphotericin B is used for the treatment of patients with progressive life-threatening fungal infections and meningitis caused by susceptible fungi. This potent drug should not be used to treat noninvasive fungal infections.

Amphotericin B is specifically intended to treat the following potentially life-threatening invasive fungal infections: cryptococcosis, aspergillosis, histoplasmosis, North America blastomycosis, candidiasis, coccidioidomycosis, zygomycosis including mucormycosis caused by *Mucor*, *Rhizopus* and *Absidia sp.* and related susceptible species of *Casidiobolus* and *Basidiobolus*, and *Sporothrix.*

Amphotericin B has been used for prophylaxis against recurrence or relapse of cryptococcosis, histoplasmosis, or coccidioidomycosis in HIV-infected individual. For HIV-infected adults and adolescent who have had documented cryptococcosis or cocccidioidomycosis, oral fluconazole is

considered the agent of first choice for lifelong suppressive therapy. Oral itraconazole or IV amphotericin B is considered an alternative agent. For HIV-infected adults and adolescent who have had documented and adequately treated histoplasmosis, oral itraconazole is considered the agent of first choice for lifelong suppressive therapy and IV amphotericin B and oral fluconazole are considered alternations.

Contraindications

Amphotericin B is contraindication in patients who are hypersensitive to the drug or any other component in the formulation, unless the condition requiring treatment is life-threatening and amenable only to amphotericin B therapy.

Warning

Amphotericin B is frequently the only effective treatment for potentially life-threatening fungal disease. In each case, its possilble life-saving benefit must be balanced against its untoward and dangerous side effects.

Nephrotoxicity: Renal damage is a limiting factor for the use of amphotericin B . Renal dysfunction usually improves upon interruption of therapy, dose reduction or increased dosing interval. Sodium loading may be effective in reducing nephrotoxicity, but this may be a problem in patients with cardiac or hepatic disease. In some patients, hydrations and sodium repletion prior to amphotericin B administration may reduce the risk of developing nephrotoxicity. Supplement alkali medication may decrease renal tubular acidosis complications.

Infusion reactions: Acute reactions including fever, shaking chills, hypotension, anorexia, vomiting, nausea, headache and tachypnea are common 1-3 hours after starting an IV infusion. These reactions are usually more severe with the first few doses of amphotericin B and usually diminish with subsequent doses. Acute infusion-related reactions can be managed by pretreatment with antihistamines and corticosteroids or by reducing the rate of infusion and by prompt administration of antihistamines and corticosteroids. Avoid rapid IV infusion because it has been associated with hypotension, hypokalemia, arrhythmias, bronchospasm and shock.

Hypersensitivity: Anaphylaxis has been reported with amphotericin B. If severe respirations distress occur, discontinue the infusion immediately. Do not give further infusions. Have cardiopulmonary resuscitation facilities available during administration.

Pregnancy: Category B. Systemic fungal infections have been successfully treated in pregnant women with amphotericin B without obvious effects to the fetus, but the number of cases reported has been small. This drug should be administered during pregnancy with caution and only if the potential benefit to the mother outweighs the potential risk to the fetus.

Lactation: It has not known whether amphotericin B is excreted in breast milk. Because of the potential for serious adverse reactions in nursing infants, decide whether to discontinue breastfeeding or discontinue treatment, taking into account the importance of the drug to the mother.

Pediatric: Safety and efficacy of amphotericin B in pediatric patients have not been established through a adequate and well-controlled studies. However, the drug has been used effectively to treat systemic fungal infection in patients without unusual adverse effects. The lowest effective dosage of the drug should be used whenever amphotericin B is used in pediatric patients.

Precautions

Monitor renal function frequently during amphotericin B therapy. It is also advisable to monitor liver function, serum electrolytes (particularly magnesium and potassium), blood counts and hemoglobin concentrations on regular basis. Use laboratory test results as guide to subsequent dose adjustments. Monitor complete blood count and prothrombin time as medically indicated.

Drug interactions

Nephrotoxic drugs

Since nephrotoxic effects may be additive, the concurrent or sequential use of amphotericin B and other drugs with similar toxic potentials (e.g., aminoglycosides, capreomycin, colistin, cisplatin, pentamidine, cyclosporine, methoxyflurane, polymyxin B, vancomycin) should be avoided, if possible. Intensive monitoring of renal function is recommended if such concomitant therapy is required.

Corticosteroids

Corticosteroids reportedly may enhance the potassium depletion caused by amphotericin B and should not be used concomitantly unless necessary to control adverse reactions to amphotericin B. If corticosteroids are used concomitantly with amphotericin B, serum electrolytes and cardiac function should be monitored closely.

Drugs affected by potassium depletion

Because amphotericin B may induce hypokalemia, the drug may predispose patients receiving cardiac glycosides to glycoside-induced cardiotoxicity and may enhance the effects of skeletal muscle relaxants (e.g., tubocurarine). Serum potassium concentrations should be monitored closely in patients receiving amphotericin B and cardiac glycosides or skeletal muscle relaxants.

Antifungal agents

A synergistic relationship has been demonstrated *in vitro* between flucytosine and amphotericin B in the inhibition of strains of *Cryptococcus neoformans*, *Candida albicans*, and *Candida tropicalis*. The suggested mechanism of the synergism is that binding of amphotericin B to sterols in cell membranes increases the permeability of the cytoplasmic membrane, thus allowing greater penetration of flucytosine into the fungal cell. There is some evidence that concomitant use of amphotericin B and flucytosine may increase the toxicity of flucytosine, possibly by increasing cellular uptake and/or by decreasing renal excretion of the drug. If flucytosine is used in conjunction with amphotericin B, especially in HIV-infected patients, blood counts should be carefully monitored. In addition, it has been suggested that serum flucytosine concentrations should be monitored in patients receiving amphotericin B and flucytosine concomitantly and flucytosine dosage reduced or the drug discontinued if accumulation occurs.

Antineoplastic agents

The manufacturers state that antineoplastic agents (e.g., nitrogen mustard, mechlorethamine) may enhance the potential for renal toxicity, bronchospasm, and hypotension in patients receiving amphotericin B; such concomitant therapy should be used with caution, particularly in immunocompromised patients.

Adverse reactions

Although some patients may be tolerate full intravenous doses of amphotericin B without difficulty, most patients will exhibit some intolerance, particularly during the initiation of therapy. Tolerance may be improved by administration of antipyretics (e.g., aspirin, acetaminophen), antihistamines, or antiemetics before the infusion and by maintaining sodium balance.

Intravenous administration of small doses of adrenal corticosteroids just prior to or during the infusion may decrease febrile reactions. Dosage and duration of such coricosteroids therapy should be kept to a minimum.

Adding a small amount of heparin to the infusion (500-200 units), rotation of the injection site, the use of pediatric scalp vein needle may lesser the incidence of thrombophlebitis. Extravasation may cause chemical irritation.

More frequent

General toxic reactions: Fever (sometimes with shaking chills usually occuring within 15-20 minutes after initiation of treatment), headache, anorexia, malaise, generalized pain, including muscle and joint pains.

Cardiovascular: Hypotension, hypertension, tachycardia, tachypnea

CNS: Confusion, headache, depression, thinking abnormal, insomnia

Dermatological: Rash, pruritus, maculopapular rash

GI: Nausea, vomiting, dyspepsia, diarrhea, cramping, epigastric pain, abdominal pain, melena, stomatitis, anorexia

GU: Hematuria

Hematologic: Normochromic, normocytic anemia, prothrombin time increased, anemia, conjugation disorder

Metabolic/Nutritional: Bilirubinemia, increased serum creatinine, acidosis, hypernatremia, hypovolemia

Renal: Decreased renal function abnormalities including: azotemia, hypokalemia, renal tubular acidosis, and nephrocalcinosis; permanent damage is often related to a large total dose (> 5 g)

Respiratory: Respiratory failure, respiratory disorder, pneumonia, dyspnea, hypoxia, epitaxis, cough increased, lung disorder, hemoptysis, hyperventilation, apnea, pleural effusion, rhinitis

Local: Venous pain at the injection site with phlebitis and thrombophlebitis

Miscellaneous: Weight loss, multiple organ failure, infection, rash, sweating, pain, chest pain, back pain, sepsis, face edema, arrhythmias, chills, eye hemorrhage, peripheral edema

Miscellaneous: Weight loss, multiple organ failure, infection, rash, sweating, pain, chest pain, back pain, sepsis, face edema, arrhythmias, chills, eye hemorrhage, peripheral edema

Less frequent (or rare)

Cardiovascular: Ventricular fibrillation, cardiac arrest, cardiac failure, shock, congestive heart failure, myocardial infarction, pulmonary edema

CNS: Peripheral neuropathy, agitation, tremor, encephalopathy, extrapyramidal syndrome, anxiety, convulsions

Dermatological: Maculopapular rash, exfoliative dermatitis, erythema multiforme, pruritus, skin disorder

Hematologic: Eosinophilia, coagulation defects, agranulocytosis, thrombocytopenia,

Hepatic: Acute liver failure, hepatitis, jaundice, hepatic failure

Administration and dosage

Amphotericin B should be administered by slow intravenous infusion. Intravenous infusion should be given over a period of approximately 2-6 hours observing the usual precautions for intravenous therapy. The recommended concentration for intravenous infusion is 0.1 mg/ml (1 mg/10 ml). Because patient tolerance varies greatly, a test dose may be preferred; 1 mg in 20 ml of 5% Dextrose delivered IV over 20-30 minutes. Record patient's temperature, pulse, respiration and blood pressure every 20-30 minutes for 2-4 hours. The recommended initial dose is 0.25-0.3 mg/kg/d prepared as 0.1 mg/ml infusion and delivered slowly over 2-6 hours. Depending on the patient's cardiorenal status, dosage may be gradually increased by 5-10 mg/d up to a total dose of 0.5-0.7 mg/kg/d. Some mycoses may total doses up to 1-1.5 mg/kg/d. Do not exceed a total daily dose of 1.5 mg/kg/d; overdose can result in cardiorespiratory arrest. Alternate daily dosing is recommended for total daily doses of 1.5 mg/kg.

For initial concentration of 5 mg/ml, rapidly inject 10 ml sterile water for infection without a bacteriostatic agent directly into lyophilized cake, using a sterile needle. Shake the vial immediately until the colloidal solution is clear. The infusion solution, providing 0.1 mg/ml, is then obtained by further dilution (1:50) with 5% Dextrose Injection of pH above 4.2. Do not dilute or reconstitute with saline solutions or mix with drugs or electrolytes. The use of any solution other than those recommended or the presence of a bacteriostatic agent may cause precipitation of amphotericin B.

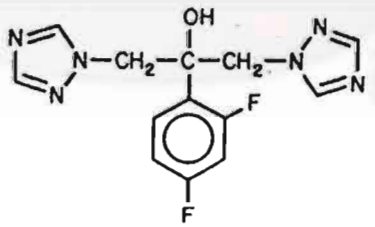
2.2 Fluconazole [32-33,38-39]

Chemistry and stability

Fluconazole is a synthetic triazole-derivative antifungal agent. The drug is structurally related to imidazole-derivative antifungal agents. Replacement of the imidazole ring with a triazole ring appearently results in increased antifungal activity and an expanded antifungal spectrum of activity. Presence of these triazole rings may contribute to fluconazole's resistence to first-pass matabolism and the drug's low lipophilicity and protein binding (Figure 2). Fluconazole is a water-soluble fluorine-substituted bis-triazole that has been shown to be effective against a variety of fungal infections in immunocompetent and immunocompromised hosts.

Fluconazole capsules should be stored in tight containers at temperature less than 30°C; fluconazole powder for oral suspension should be stored at a temperature less than 30°C. After reconstitution, refrigeration of fluconazole oral suspension is not necessary and freezing of the suspension should be avoided. Commercially available fluconazole provided in glass bottles should be stored at 5-30°C and protected from freezing.

Figure 2: Chemical structure of fluconazole



Mechanism of action

Fluconazole usually is fungistatic in action. Like imidazoles, fluconazole exerts its antifungal activity by altering cellular membranes resulting in increased membrane permeability, leakage of essential elements (e.g., amino acids, potassium) and impaired reuptake of precursor molecules (e.g. purine and pyrimidine precursors to DNA). This drug is highly selective inhibition of fungal cytochrome P-450 and sterol 14-α-methylase in susceptible fungi, which leads to accumulation of C-14 methylated sterols and decreased concentrations of ergosterol. However, it is significantly less potent in the inhibition of the mammalian enzyme, and thus is more specific inhibitor of fungal cytochrome P-450 system. The drug does not appear to have any effect on cholesterol synthesis in mammalian liver homogenates.

Microbiology

Fluconazole is active against many fungi, including yeasts and dermatophytes. Fluconazole does not appear to have antibacterial activity. Fluconazole exhibits *in vitro* activity against *Cryptococcus neoformans* and *Candida* sp. Fungistatic activity has also been demonstrated in normal and immunocompromised animal models for systemic and intracranial fungal infections due to *C. neoformans* and for systemic infections due to *C. albicans*. Development of resistance to fluconazole has not been studied; however, there have been reports of cases of superinfection with *Candida* species other than *C. albicans*, which are often inherently nonsusceptible to fluconazole. Such cases may require alternative antifungal therapy.

Pharmacokinetics

Absorption

The pharmacokinetics of fluconazole are similar following IV or oral administration. The drug is rapidly and almost completely from the gastrointestinal (GI) tract, and there is no evidence of first-pass metabolism. Oral bioavailability of fluconazole exceeds 90% in healthy, fasting adults; peak plasma concentrations of the drug generally are attained within 1-2 hours after oral administration. The rate and extent of GI absorption of fluconazole are not affected by food. Unlike some imidazole-derivative antifungal agents GI absorption of fluconazole does not appear to be affected by gastric pH. Peak plasma fluconazole concentrations and the areas under the concentration-time curves (AUCs) increase in proportion to the dose over the oral dosage range of 50-400 mg. Steady-state plasma concentrations of fluconazole are attained within 5-10 days following oral doses of 50-400 mg given once daily.

The manufacturer states that when fluconazole therapy is initiated with a single loading dose equal to twice the usual daily dosage and followed by the usual dosage given once daily thereafter, plasma concentrations of the drug reportedly approach steady state by the second day of therapy

Distribution

Fluconazole is widely distributed into body tissue and fluids following oral or IV administration. In adult humans with normal renal function, concentrations of the drug attained in urine and skin may be 10 times higher than concurrent plasma concentrations; concentrations attained in saliva, sputum, nails, blister skin, and vaginal tissue are approxiamately equal to concurrent plasma concentrations.

Fluconazole, unlike some azole-derivative antifungal agents (e.g., itraconazole, ketoconazole), distributes readily into CSF following oral or IV administration; CSF concentrations of fluconazole may be 50-94% of concurrent plasma concentrations regardless of the degree of meningeal inflammation. The apparent volume of distribution of fluconazole approxiamates that of total body water and has been reported to be 0.7-1 L/kg. Fluconazole is only 11-12% bound to plasma proteins.

Metabolism and elimination

Fluconazole is eliminated mainly by renal rather than hepatic mechanisms. The plasma elimination half-life of fluconazole in adults with normal renal function is approximately 30 hours (range 20-50 hours). In patients with impaired renal function, plasma concentrations of fluconazole are higher and the half-life prolonged; elimination half-life of the drug is inversely proportional to the patient's creatinine clearance. In additional, there is limited evidence that elimination of the drug may be impaired in geriatric patients. The elimination half-life of fluconazole reportedly is not affected by impaired hepatic function.

In healthy adults, fluconazole is eliminated principally by renal excretion. Renal clearance of the drug averages 0.27 ml/min/kg in adults with normal renal function. Approximately 60-80% of a single oral or IV dose of fluconazole is excreted in urine unchanged, and about 11% is excreted in urine as metabolites. Small amounts of the drug are excreted in feces. Fluconazole is removed by hemodialysis and peritoneal dialysis. The amount of the drug removed during hemodialysis depends on several factors. A 3-hour period of hemodialysis generally decreases plasma concentration of the drug by 50%.

Indications

- Candidiasis

Treatment - Oropharyngeal or esophageal candidiasis, vaginal candidiasis, candidal urinary tract infections, peritonitis and systemic candidal infections including candidemia, disseminated candidiasis and pneumonia

Prophylaxis - To decrease the incidence of candidiasis in patients undergoing bone marrow transplantation who receive cytotoxic chemotherapy or radiation therapy

- Cryptococcal meningitis: Treatment of cryptococcal meningitis

Contraindications

Fluconazole is contraindicated in patients with known hypersensitivity to the drug or any ingredient in the respective formulation. Although information concerning cross-sensitivity between fluconazole and other triazole or imidazole antifungal agents is not available, the manufacturer states that fluconazole should be used with caution in individuals hypersensitivity to other azoles.

Warnings

Hepatic injury: Fluconazole has been associated with rare cases of serious hepatic toxicity and often while taking multiple concomitant medications. If abnormal liver function test results occur during fluconazole therapy, the patients should be monitored for the development of more severe hepatic injury. Fluconazole therapy should be discontinued if signs and symptoms consistent with liver disease develop. In each of these cases, liver function returned to baseline on discontinuation of fluconazole.

Dermatological changes: Because potentially fatal exfoliative skin disorders have been reported rarely in patients with a serious underlying disease receiving fluconazole, the possibility that these effects can occur should be considered. Immunocompromised patients who develop rash during fluconazole therapy should be monitored closely and the drug discontinued if the lesions progress.

Carcinogenicity: There was an increased incidence of hepatocellular adenomas in male rats receiving an oral fluconazole dosage of 5 or 10 mg/kg daily

Pregnancy: There are no adequate and controlled studies to date using fluconazole in pregnant women. The drug should be used during pregnancy only when the potential benefits justify the possible risks to the fetus.

Lactation: Fluconazole is excreted in breast milk at concentrations similar to plasma. Therefore, the use of fluconazole in nursing mother is not recommended.

Drug interactions

While fluconazole can alter the pharmacokinetics of certain drugs that undergo hepatic metabolism, the magnitude of such alterations appears to be less than those associated with ketoconazole.

Coumarin anticoagulants

Increased prothrombin time has been reported in patients receiving fluconazole concomitantly with a coumarin anticoagulant. Prothrombin time should be monitored carefully when fluconazole is used concomitantly with a coumarin anticoagulant.

Cyclosporine

Concomitant administration of fluconazole and cyclosporine may result in increased plasma cyclosporine concentrations, especially when the drugs are used in renal transplant recipients. Plasma cyclosporine concentrations should be monitored carefully in patients receiving fluconazole concomitantly, and dosage adjusted accordingly.

Astemizole and terfenadine

The possibility that fluconazole may interact with astemizole and terfenadine, resulting in potentially serious adverse cardiovascular effects, should be considered. Prolongation of the QT interval and, rarely, serious cardiovascular effects, including arrhythmias, arrest, palpitation, syncope, and death, have been reported in patients receiving the structurally similar antifungal ketoconazole concomitantly with recommended dosages of terfenadine.

Drugs affecting gastric acidity

Cimetidine resulted in reduction in AUCs of fluconazole. Studies in fasting, healthy adults indicate that GI absorption of fluconazole is not affected substantially by concomitantly administration of drugs that decrease gastric acid output or increase gastric pH. Administration of antacids containing aluminum hydroxide or magnesium hydroxide either with or

immediately prior to a single 100 mg oral dose of fluconazole had no effect on absorption or elimination of the antifungal agents.

Oral contraceptives

Concomitant administration of fluconazole and oral estrogen-progestin contraceptives reported does not produce clinically important effects on the pharmacokinetics of the contraceptives.

Phenytoin

Concomitant administration of fluconazole and phenytoin has resulted in increased plasma phenytoin concentrations and AUCs and has resulted in phenytoin toxicity. Plasma phenytoin concentrations should be monitored carefully and dosage of the anticonvulsant adjusted accordingly whenever fluconazole is initiated or discontinued. Phenytoin and fluconazole should be used concomitantly with caution.

Rifampin

Rifampin enhances the metabolism of fluconazole. Concomitant administration of the drugs has resulted in a decrease in plasma fluconazole concentration and a 25% decrease in the AUCs and a 20% decrease in the plasma half-life of the antifungal. Consideration should be given to increasing the dosage of fluconazole when the drug is administered concomitantly with rifampin.

Sulfonylurea antidiabetic agents

Administration of fluconazole in individual receiving tolbutamide, glyburide, or gliplizide has resulted in increased plasma concentrations and reduced metabolism of the antidiabetic agents. If fluconazole is used concomitantly with tolbutamide, glyburide, glipizide, or any other oral sulfonylurea antidiabetic agent, blood glucose concentrations should be monitored carefully and dosage of the antidiabetic agent adjusted as necessary.

Thiazide diuretic

It has been suggested that the thiazide diuretic decreased renal clearance of fluconazole by about 20%; however, adjustment of fluconazole dosage does not appear to be necessary during combined therapy with the drugs.

Zidovudine and other dideoxynucleoside antiviral agents

Concomitant administration of fluconazole appears to interfere with the metabolism and clearance of zidovudine. Although the clinical importance of this effects is unknown, it has suggested that patients receiving concomitant

zidovudine and fluconazole therapy be monitored closely for zidovudine-associated adverse effects.

Adverse reactions

Fluconazole generally is well tolerated. Adverse effects have been reported in about 5-30% of patients receiving fluconazole for 7 days or longer and have been severe enough to require discontinuance of the drugs in about 1-2.8% of patients.

GI effects

Mild to moderate nausea, vomiting, abdominal pain, and diarrhea have been reported in about 1.5-8.5% of patients receiving fluconazole. Only rarely were such adverse GI effects severe enough to require discontinuance of the drug.

Dermatological effects

Rash, including diffuse rash accompanied by eosinophilia, and pruritus have been reported in up to about 5% of patients receiving fluconazole. Exfoliative skin disorders have been reported rarely in patients with serious underlying disease.

Hepatic effects

Mild transient increases (1.5-3 times the upper limit of normal) in serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, and bilirubin have been reported in about 5-7% of patients receiving fluconazole. In most reported cases, concentrations returned to pretreatment levels either during or after fluconazole therapy and were not associated with hepatotoxicity. The incidnce of increased serum transaminase concentrations was greatest in patients receiving fluconazole concomitantly with rifampin, phenytoin, isoniazid, valproic acid, and/or oral sulfonylurea hypoglycemic agents. Transient hepatic reactions, including hepatitis and jaundice, have been reported in patients without identifiable risk factors; following discontinuance of fluconazole, liver function returned to baseline in these patients.

Central nervous system effects

Dizziness and headache have been reported in up to about 2% of patients receiving fluconazole. Somnolence, delirium/coma, dysethesia, psychiatric disturbances, malaise, paresthesia of hands and feet, and fatigue have been reported rarely.

Hematologic effects

Eosinophilia has been reported in some patients receiving fluconazole. Anemia, leukopenia, neutropenia, and thrombocytopenia also have been reported. In at least one AIDS patient, thrombocytopenia occurred during fluconazole therapy and resolved following discontinuance of the drug.

Endocrine effects

Studies using usual dosages of fluconazole have not shown evidence of adverse effect related to possible inhibition of testosterone or steroid synthesis.

Other adverse effects

Fever, edema, pleural effusion, oliguria, hypotension, anaphylaxis, arthralgia/myalgia, and finger stiffness have been reported rarely in patients receiving fluconazole. Hypokalemia, which required potassium replacement therapy and/or discontinuance of fluconazole, has occured occasionally, including in several neutropenic patients with acute myeloid leukemia. Increased serum creatinine and BUN concentrations also have been reported .

Administration and dosage

Fluconazole is administered orally or by IV infusion. Fluconazole may be given orally without regard to meals. Since absorption of fluconazole from the GI tract is rapid and almost complete, IV therapy with the drug generally is reserved for patients who do not tolerate or are unable to take the drug orally.

Oral and IV dosage of fluconazole are identical. Dosage of the drug should be based on the type and severity of the infection, identity of the causative organism, and the patients's renal function and response to therapy.

Single dose: Vaginal candidiasis - 150 mg as a single oral dose

Multiple dose: For infections other than vaginal candidiasis, base the daily dose on the infecting organism and patient response to therapy. Continue treatment until clinical parameters or lab tests indicate that active fungal infection has subsided. An inadequate treatment period may lead to recurrence of active infection.

Oropharyngeal candidiasis - 200 mg on first day, follow by 100 mg once daily. Clinical evidence of orophryngeal candidiasis generally resolves within several days, but continue treatment for 2 weeks to decrease likelihood of relapse.

Esophageal candidiasis - 200 mg on the first day, followed by 100 mg once daily. Doses up to 400 mg/day may be used, based on the patient's response. Treat patients with esophageal candidiasis for a minimum of 3 weeks and for at least 2 weeks following resolution of symptoms.

Candidiasis, others - For candidal urinary tract infections (UTIs) and peritonitis, 50-200 mg/day has been used. For systemic candidal infections (including candidemia, disseminated candidiasis and pneumonia), optimal dosage and duration have not been determined, although doses up to 400 mg/day have been used.

Prevention of candidiasis in bone marrow transplant - 400 mg once daily. In patients who are anticipate to have severe granulocytopenia (< 500 neutrophils/mm^3), start fluconazole prophylaxis several days before anticipate onset of neutropenia, and continue 7 days after neutrophil count rises above 1,000 cells/mm^3.

Cryptococcal meningitis - 400 mg on the first day, followed by 200 mg once daily. A dosage of 400 mg once daily may be used, based on the patient's response to therapy. The duration of treatment for initial therapy of cryptococcal meningitis is continuously until the cerebrospinal fluid becomes cultures negative. The dosage of fluconazole for suppression of relapse of cryptococcal meningitis in patients with AIDS is given 200 mg once daily indefinitely.

Dosage in renal impairment: In patients with impaired renal function, dosage of fluconazole must be modified in response to the degree of impairment and should be based on the patient's measured or estimated creatinine clearance. The patient's creatinine clearance (Clcr) can be estimated by using the following formula:

$$\text{Clcr male} = \frac{(140\text{-age}) \times \text{weight}}{72 \times \text{serum creatinine}}$$

$$\text{Clcr female} = 0.85 \times \text{Clcr male}$$

(when age is in years, weight is in kg, and serum creatinine is in mg/dl)

The manufacturer recommends that adults with impaired renal function should receive an initial 50-400 mg loading dose of fluconazole followed by daily maintenance doses based on creatinine clearance as followed:

Creatinine Clearance (ml/min)	% of Usual Daily Dose
> 50	100%
21 - 50	50%
11 - 20	25%
Patients receiving regular hemodialysis	One recommended dose after each dialysis

CHAPTER III

PATIENTS AND METHODS

The study was conducted from October 1999 to March 2000 at Bamrasnaradura Hospital, Nonthaburi, Thailand.

Patients

Study population

This study was designed as a prospective randomized double-blind trial to compare the efficacy and safety of amphotericin B with or without fluconazole for treatment of cryptococcal meningitis in patients with AIDS. The study protocol was reviewed and approved by the institutional review board at study site. The subjects of this study were selected from a group of AIDS patients associated with cryptococcal meningitis who visited the Ambulatory Care Unit at Bamrasnaradura Hospital. Written informed consent had to be given by the patients or his legal guardian. Patients would be enrolled on the basis of a positive india ink stain of the cerebrospinal fluid, while culture results were pending. Following enrollment, confirmation of cryptococcal meningitis was required by a positive cerebrospinal fluid culture for *Cryptococcus neoformans*. The criteria for enrollment included:

Inclusion criteria

1. HIV-infection documented by a positive test for HIV antibody or a previous AIDS-defining opportunistic infections.
2. An age of 18 years or older.
3. A first episode of cryptococcal meningitis documented by clinical and CSF findings positive india ink compatible with diagnosis of cryptococcal meningitis plus a positive cerebrospinal fluid culture for *C. neoformans*.

Exclusion criteria

1. They were pregnant or lactating.
2. They had a known allergy to polyene or azoles antifungal.
3. They had already received a total dose of amphotericin B more than 1 mg/kg within 14 days or more than 1,200 mg of fluconazole, itraconazole, ketoconazole within 3 days before entry.

4. They were diagnosed another concurrent central nervous system (CNS) infections.

5. They were unable to take medications by mouth or nasogastric tube.

6. They had a significantly impaired hepatic function (aminotransferase concentrations more than five times the upper limit of normal).

7. They had a significantly impaired renal function (concentration above 3.5 mg/dl or creatinine clearance rate of less than 20 ml/min).

8. Concomitant therapy with anticoagulant, oral hypoglycemic agents, barbiturates, phenytoin, rifampicin, and other drugs, which had significantly interaction with fluconazole, were not allowed.

Methods

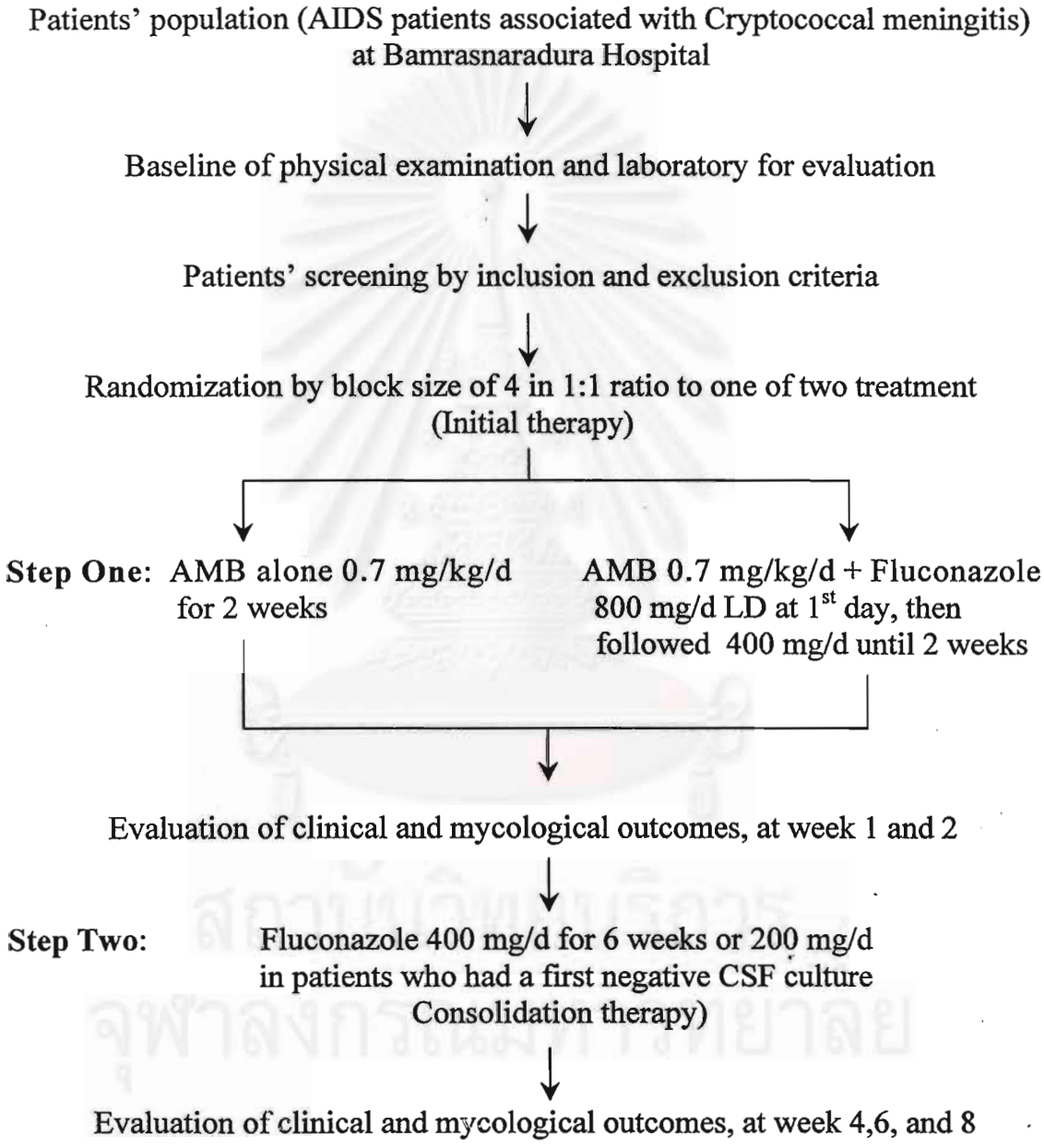
Study design and treatment

The study was conducted in two steps (Figure 3). In step one, patients were randomly assigned by block size of 4 to receive amphotericin B (0.7 mg/kg/d) with either fluconazole (administered orally in a single dose, 800 mg loading dose on the first day and in a dose of 400 mg daily thereafter) or placebo for the initial two weeks of therapy. In step two, all patients completing initial therapy were given fluconazole alone (400 mg/d) for six weeks. The dosage of fluconazole was reduced by a half (200 mg/d) for patients who had a first negative cerebrospinal fluid culture for *C. neoformans* and continued this dose as the maintenance therapy to prevention of relapse. After 8 weeks, the patients who had positive CSF culture were received fluconazole alone (400 mg/d) until conversion to the first negative CSF culture. The dosage of fluconazole was reduced to 200 mg daily and continued this dose as the maintenance therapy.

Monitoring and evaluation

Clinical and laboratory evaluation were performed at base line, weekly for the first 2 weeks and then every 2 weeks until 8-week study period was completed. Each evaluation, assessment of signs and symptoms were performed and any adverse events were assessed and recorded. Patients were evaluated for vital signs, the presence of meningeal symptoms, and Glasgow Coma Scale. During this period an additional neurological and general physical examination, as well as determination of the Karnofsky Performance Scale, was performed weekly for the first 2 weeks and then every 2 weeks until 8-week study period. Serum electrolytes were measured as well as the renal and hepatic function were tested. The complete blood counts were obtained to ensure the patients' safety.

Figure 3: Flow chart of the study



Lumbar punctures for analysis of cerebrospinal fluid and the culture were performed at base line, and at the end of 2, 4, 6, and 8 weeks, or until the cerebrospinal fluid showed two consecutive negative cultures. Adverse events were assessed by patient interviews and measurement of complete blood counts, serum electrolytes, renal and hepatic function tests weekly for the first two weeks and every 2 weeks until week 8, then compared with normal data or values at baseline.

Outcomes

Clinical and mycological outcomes were evaluated separately and together as a composite outcome.

Clinical outcomes were categorized into:

1. Improvement, defined as signs and symptoms of meningitis were subsided or no worse.
2. Not improvement, defined as signs and symptoms of meningitis were worsened.

Mycological outcomes were classified as:

1. Conversion, defined as conversion of positive to negative CSF cultures of *C. neoformans*.
2. Persistence, defined as persistence of positive CSF cultures.

The following definitions of treatment outcomes were used:

1. Successful, defined as the patient had clinical improvement or complete resolution of signs and symptoms together with two consecutive negative cultures of CSF samples obtained at least one week apart.
2. Unsuccessful or treatment failure was classified as:
   - Quiescent disease or partial response, defined as clinical stable, progressive improvement or resolution of symptoms but persistently positive cultures of CSF or only one negative CSF cultures at the end of the 8-week treatment period.
   - Progressive disease, defined as progressive clinical deterioration in the presence of persistently positive cultures or died of progressive cryptococcal meningitis.
   - Toxic reaction, defined as a serious or intolerance adverse event that was judged to be least possibly related to this study drugs by the investigators and that led to permanent discontinuation of the study drug.
   - Inevaluable, defined as died due to another causes during therapy.

- Relapse, defined as recurrence of clinical symptoms in the presence of a positive CSF culture or clinical deterioration associated with repeatedly positive CSF culture after initial conversion.

Statistical analysis

The primary efficacy analysis in this study was performed by comparing the rates of successful treatment between the two groups. We assumed that if amphotericin B combined with fluconazole is 30 percent or more effective than amphotericin B alone, as judged by successful sterilization of CSF cultures in initial therapy of cryptococcal meningitis in patients with AIDS, then amphotericin B with fluconazole should be considered as a better regimen for the treatment of cryptococcal meningitis. Using one-sided test, an alpha level of 0.05, and a power of 80 percent to detect a true difference of 30 percent in the mycological outcomes between the two groups, the analysis would required at least 30 patients per group.

Information obtained from the subjects and laboratories were recorded in case report form. The data were analyzed by descriptive statistics, compared by chi-square test or Fisher's exact test for categorical variable and by student's t-test for continuous variables. The Kaplan-Meier method was used to estimate the time to the first negative CSF cultures and the two treatment groups were compared by the log rank test. All analysis of data were performed on an intention-to-treat basis.

CHAPTER IV

RESULTS AND DISCUSSION

1. Study population

Between October 1999 and March 2000, 74 patients with AIDS-associated cryptococcal meningitis (confirmed by a positive india ink and CSF culture) were enrolled in this study during their first episode of cryptococcal meningitis, at Ambulatory Care Unit, Bamrasnaradura Hospital. All of the patients gave their consent to participate in this study. Of the 74 patients enrolled, 38 patients were randomly assigned to receive amphotericin B plus fluconazole (AMB + FLU) while 36 patients were assigned to receive amphotericin B alone (AMB). Two patients in the later group were referred to other hospital. Therefore, only 72 patients were included in the further analysis (38 patients in AMB + FLU group, 34 patients in AMB group).

Demographic data

Of the 72 patients with AIDS-associated cryptococcal meningitis, 56 patients (77.8%) were male. With a range of 21-62 years, most of patients (41.7%) were within 20-29 years with the mean age equal to 32.5 ± 8.5 years (mean ± S.D.). The top three occupations of the patients were employee (33.4%), unemployed (31.9%), and government officer (13.9%), respectively. Fifty patients (69.4%) were married while 18 patients (25%) and 4 patients (5.6%) were single and divorced, respectively. Risk factors of HIV-infection included sexual contact in 69 patients (95.8%), combined sexual contact with intravenous drug use (IVDU) 2 patients (2.8%) and unknown risk factor in 1 patient (1.4%). Demographic data were shown in Table 1.

Table 1: Demographic data of patients with AIDS-associated cryptococcal meningitis

Data	No. (%) of patients		
	AMB + FLU (n=38)	**AMB (n=34)**	**TOTAL (n=72)**
Sex			
1. Male	27(71.1)	29(85.3)	56(77.8)
2. Female	11(28.9)	5(14.7)	16(22.2)
Age (yrs)			
1. 20 – 29	13(34.2)	17(50.0)	30(41.7)
2. 30 – 39	19(50.0)	9(26.5)	28(38.9)
3. ≥ 40	6(15.8)	8(23.5)	14(19.4)
Mean ± S.D.	32.4 ± 8.3	32.7 ± 8.8	32.5 ± 8.5
Range	21-62	21-55	21-62
Occupation			
1. Unemployed	15(39.5)	8(23.5)	23(31.9)
2. Employee	10(26.4)	14(41.1)	24(33.4)
3. Commercial	4(10.5)	2(5.9)	6(8.3)
4. Agriculturist	4(10.5)	2(5.9)	6(8.3)
5. Government officer	4(10.5)	6(17.7)	10(13.9)
6. Others	1(2.6)	2(5.9)	3(4.2)
Marital status			
1. Married	27(71.7)	23(67.6)	50(69.4)
2. Single	8(21.0)	10(29.4)	18(25.0)
3. Divorced	3(7.9)	1(3.0)	4(5.5)
Risk factors			
1. Sexual contact	36(94.8)	33(97.1)	69(95.8)
- Homosexual	2(5.3)	3(8.8)	5(6.9)
- Heterosexual	34(89.5)	30(88.3)	64(88.9)
2. Sexual contact + IVDU	1(2.6)	1(2.9)	2(2.8)
3. Unknown	1(2.6)	0(0)	1(1.4)

Clinical characteristics

The presenting baseline clinical characteristics of the patients were summerized in Table 2. Before treatment, the most common presenting symptoms was headache (97.2%), nausea/vomiting (84.7%), and fever (59.7%). Neck stiffness was observed in 25 patients (34.7%). Impaired consciousness was noted in 11 patients (15.3%), visual change in 11 patients (15.3%), and hearing loss in 11 patients (15.3%). Other signs and symptoms such as seizure, papilledema, and photophobia were found in less than 10 %. The extrameningeal involvements are common in AIDS patients with cryptococcal meningitis, usually is found between 20% and 60%[17-18,40] and may often lead to diagnosis of meningitis. In this study, the occurrence of concomitant extrameningeal disease were found to be less than 20%. Ten patients (13.9%) had lung involvement, all presented with cough . However, only 3 patients who had pulmonary cryptococcosis were confirmed by sputum culture positive with *C. neoformans*. Skin lesions with *C. neoformans* was found in only 1 patient. Lymphadenopathy, hepatomegaly, and splenomegaly were presented in visceral cryptococcosis.

Baseline clinical signs and symptoms of patients were similar to those in the other studies.[9-10,26-27,40-41] Almost all of the signs and symptoms in the two groups were not significantly different ($P > 0.05$) except fever, visual changes, and hearing loss were found to be more in the AMB + FLU group as compared to the AMB group and were statistically significant different ($P < 0.05$).

Glasglow Coma Scale (GCS) and Karnofsky Performance Scale (KPS) were used to evaluate clinical signs and symptoms of patients. The mean GCS and KPS were 14.5 ± 1.5 and 62.4 ± 13.6, respectively. Most patients were not coma and required occasional assistance but were able to care for most of their own needs. Fifty-six patients (77.8%) and 16 patients (22.2%) were classified in mild to moderate group and severe group, respectively.

The mean time of symptoms before admission was 12.3 ± 11.2 days (range, 1-60 days). Sixty patients (83.3%) had symptoms less than 14 days before admission while 12 patients (16.7%) had symptoms longer than 14 days before admission. These results were agreed with those reported in other studies which indicated that most of the patients generally presented to the hospital during 2-4 weeks after the symptoms.[2-3,17-18,20-21,42-43]

Table 2: Baseline clinical characteristics of patients with AIDS-associated cryptococcal meningitis

Characteristics	No. (%) of patients		
	AMB + FLU (n=38)	AMB (n=34)	TOTAL (n=72)
Signs and symptoms			
1. Headache	37(97.4)	33(97.1)	70(97.2)
2. Fever	27(71.1)	16(47.1)	43(59.7)
3. Nausea / Vomiting	33(86.8)	28(82.4)	61(84.7)
4. Neck stiffness	11(28.9)	14(41.2)	25(34.7)
5. Cough	6(15.8)	4(11.8)	10(13.9)
6. Dyspnea	3(7.9)	3(8.8)	6(8.3)
7. Impaired consciousness	6(15.8)	5(14.7)	11(15.3)
8. Seizure / Convulsion*	3(7.9)	1(3.0)	4(5.6)
9. Papilledema**	3(8.1)	3(9.1)	6(8.6)
10. Visual change**	9(24.3)	2(6.1)	11(15.3)
11. Photophobia*	2(5.4)	1(2.9)	3(4.2)
12. Hearing loss*	9(24.3)	2(5.9)	11(15.3)
13. Skin lesions*	0(0)	1(2.9)	1(1.4)
14. Lymphadenopathy*	5(13.5)	7(20.6)	12(16.9)
15. Hepatomegaly	5(13.2)	2(5.9)	7(9.7)
16. Splenomegaly	1(2.6)	2(5.9)	3(4.2)
Glasglow Coma Scale			
Mean ± S.D.	14.7 ± 0.8	14.4 ± 1.9	14.5 ± 1.5
Range	11-15	7-15	7-15
Karnofsky Performance Scale			
Mean ± S.D.	63.4 ± 13.2	61.8 ± 14.2	62.4 ± 13.6
Range	20-80	20-90	20-90
Severity of disease			
1. Mild to moderate	31(81.6)	25(73.5)	56(77.8)
2. Severe	7(18.4)	9(26.5)	16(22.2)
Time before admission			
1. < 7	10(26.3)	9(26.5)	19(26.4)
2. 7-14	20(52.6)	21(61.8)	41(56.9)
3. > 14	8(21.1)	4(11.7)	12(16.7)
Mean ± S.D.	13.5 ± 13.5	11.0 ± 8.1	12.3 ± 11.2
Range	1-60	2-30	1-60

* Data were analyzed from 37 patients in AMB+FLU group and 34 patients in AMB group

** Data were analyzed from 37 patients in AMB+FLU group and 33 patients in AMB group

Concomitant diseases

Table 3 presented concomitant diseases or the coexisting opportunistic infections found in patients with AIDS-associated CM. Approximately, half of the patients had concomitant diseases. About 75% of them suffered from dual infections in addition to cryptococcal meningitis. Concomitant diseases were found in 22 patients (57.9%) of the AMB + FLU group and only 13 patients (38.2%) of the AMB group (P = 0.10).

Oral candidiasis (22.2%), herpes simplex (5.6%), and tuberculosis (4.1%) were the common coexisting opportunistic infections associated with cryptococcal meningitis, same as those reports from South Africa[44] but unlike the reports from the West, where the most coexisting diseases is *Pneumocystis carinii* pneumonia (PCP).[2,40] PCP and cytomegalovirus infection were the cause of death in one and two patients, respectively.

Table 3: Concomitant diseases or the coexisting opportunistic infections

Diseases	No. (%) of patients		
	AMB + FLU (n=38)	**AMB (n=34)**	**TOTAL (n=72)**
No concomitant diseases	**16(42.1)**	**21(61.8)**	**37(51.4)**
Concomitant diseases	**22(57.9)**	**13(38.2)**	**35(48.6)**
1. Oral candidiasis (OC^+)	8(21.1)	8(23.5)	16(22.2)
2. Herpes simplex (HSV)	4(10.5)	0(0)	4(5.6)
3. Tuberculosis	2(5.3)	1(2.9)	3(4.1)
4. *Pneumocystis carinii* pneumonia (PCP)	2(5.3)	0(0)	2(2.8)
5. Cytomegalovirus infection (CMV)	1(2.6)	1(2.9)	2(2.8)
6. Pruritic papular eruption	1(2.6)	0(0)	1(1.4)
7. HSV + OC^+	2(5.3)	0(0)	2(2.8)
8. HSV + PCP	0(0)	1(2.9)	1(1.4)
9. HSV + CMV	1(2.6)	0(0)	1(1.4)
10. Others	1(2.6)	2(6.0)	3(4.1)

Laboratory findings

Cryptococcus neoformans has become the most important cause of fungal meningitis worldwide. Cryptococcal infection in patients with AIDS is usually associated with severe immunodeficiency. The CD_4^+ cell count is almost consistently below 100 cells/mm^3 and the cost of these tests are very expensive.[45] Thus, CD_4^+ cell count are not routinely determined for patients in this study. Baseline laboratory findings of patients with AIDS-associated cryptococcal meningitis were shown in Table 4. Serum electrolytes, renal and hepatic function tested, complete blood cell count and lumbar puncture were performed at baseline for all the patients while extrameningeal examination were performed only in patients whose clinical features were indicated.

Low hematocrit level in both groups was a common finding in patients with AIDS. The mean hematocrit level was 31.0 ± 6.5 percentage.White blood cell count showed wide variation among patients in both groups with ranged between 1,000 and 23,300 cells/mm^3 and the mean equal to 5,100 ± 3,400 cells/mm^3 which is in the normal value range.

Lumbar puncture for analysis of biochemical CSF components and culture were performed in all patients. CSF components are nonspecific and may be show normal value in the cryptococcal meningitis patients.[23] Nevertheless, abnormal CSF components could be used as indicator to diagnosis of cryptococcal meningitis Abnormal CSF components consists of high protein level (> 45 mg/dl), low glucose level (< 40 mg/dl), and low CSF white blood cell count (< 20 cells/mm^3).[14,20,23-24,45-46] In this study, opening pressure (OP) was higher than 200 mmH_2O in 66 patients (91.7%). Most of the patients in both groups had OP in the 200-450 mmH_2O range. The mean OP was 383.8 ± 141.1 mmH_2O in the AMB + FLU group and 391.5 ± 155.6 mmH_2O in the AMB group which were not significant difference (P = 0.83). The average OP in both groups was near 400 mmH_2O. Opening pressure higher than 600 mmH_2O were found in 15 patients and might affect the clinical outcome. Approximately 43% (3 patients in the AMB + FLU group and 4 patients in the AMB group) of patients with OP more than 600 mmH_2O died during therapy. The high OP was related to poor prognosis of this disease same as in other reports.[5,17,28] During therapy, raised in intracranial pressure (ICP) is a sign that should be concerned in patients with cryptococcal meningitis and OP should be measured routinely in all patients.[2]

Table 4: Baseline laboratory findings of patients with AIDS-associated cryptococcal meningitis

Characteristics	No. (%) of patients			
	AMB + FLU (n=38)	AMB (n=34)	TOTAL (n=72)	P-value
Complete blood count				
1. Hematocrit (%)				
Mean ± S.D.	30.0 ± 6.0	32.2 ± 7.0	31.0 ± 6.5	0.15
Range	15-43	17-48	15-48	
2. White blood cell ($X\ 10^3$ cells/mm^3)				
Mean ± S.D.	4.7 ± 2.4	5.6 ± 4.1	5.1 ± 3.4	0.23
Range	1.1-14.8	1.0-23.3	1.0-23.3	
CSF components				
1. Opening pressure (mmH_2O)				
< 200	3(7.9)	3(8.8)	6(8.3)	
200-450	21(55.3)	19(55.9)	40(55.6)	
> 450	14(36.8)	12(35.3)	26(36.1)	
Mean ± S.D.	383.8 ± 141.1	391.5 ± 155.6	387.4 ± 147.1	0.83
Range	125-600	180-600	125-600	
2. WBC (cells/mm^3)				
< 20	28(73.7)	27(79.4)	55(76.4)	
≥ 20	10(26.3)	7(20.6)	17(23.6)	
Mean ± S.D.	25.5 ± 44.2	20.2 ± 40.3	23.0 ± 42.2	0.60
Range	0-250	0-195	0-250	
3. Protein (mg/dl)				
≤ 45	16(42.1)	21(61.8)	37(51.4)	
> 45	22(57.9)	13(38.2)	35(48.6)	
Mean ± S.D.	58.7 ± 34.5	46.7 ± 19.7	53.0 ± 29.0	0.08
Range	1.7-160	23.6-98.5	1.7-160	
4. Glucose (mg/dl)				
< 40	21(55.3)	10(29.4)	31(43.1)	
≥ 40	17(44.7)	24(70.6)	41(56.9)	
Mean ± S.D.	37.4 ± 17.5	45.6 ± 15.2	41.3 ± 16.9	0.04
Range	1-74	5-70	1-74	

Table 4: Baseline laboratory findings of patients with AIDS-associated cryptococcal meningitis. (continue)

Characteristics	No. (%) of patients			
	AMB + FLU (n=38)	**AMB (n=34)**	**TOTAL (n=72)**	**P-value**
5. India-ink positive	38(100.0)	34(100.0)	72(100.0)	1.00
6. No. of fungus cell				
Mean ± S.D.	36.1 ± 34.4	27.2 ± 29.4	31.9 ± 32.2	0.24
Range	1-100	1-100	1-100	
7. CSF culture positive	38(100.0)	34(100.0)	72(100.0)	1.00
Extrameningeal culture positive	**3(7.8)**	**4(12.0)**	**7(9.8)**	
1. Sputum	1(2.6)	0(0)	1(1.4)	
2. Lymph node	0(0)	1(3.0)	1(1.4)	
3. Skin lesions	0(0)	1(3.0)	1(1.4)	
4. Stool	1(2.6)	0(0)	1(1.4)	
5. Sputum + stool	0(0)	1(3.0)	1(1.4)	
6. Sputum + duodenal biopsy	0(0)	1(3.0)	1(1.4)	
7. Pus from ears	1(2.6)	0(0)	1(1.4)	
Negative / Not done	**35(92.2)**	**30(88.0)**	**65(90.2)**	**0.70**

Analysis of biochemical CSF components showed that most of the patients (55 patients, 76.4%) had white blood cell (WBC) less than 20 cells/mm^3 which suggested poor prognosis. This part of the results was the same as in the other reports.[2,5,9,17,45] Comparison of the mean CSF WBC between the AMB + FLU group and the AMB group showed no significant difference (P = 0.60).

Abnormal protein level (>45 mg/dl) was found in the higher percentage in the AMB + FLU group (22 patients, 57.9%) as compared to the AMB group. The mean protein level were 58.7 ± 34.5 and 46.7 ± 19.7 mg/dl in the AMB + FLU group and the AMB group, respectively. However, there were no significant difference between the two groups (P = 0.08). Abnormal glucose level (< 40 mg/dl) was found more often in the AMB + FLU group (21 patients, 55.3%) as compared to the AMB group. The lower mean glucose level in the AMB + FLU group was 37.4 ± 17.5 mg/dl. When the means glucose level were compared, there were significant difference between the two groups (P = 0.04) which may affected the outcome of therapy.

All patients showed a positive india ink before enrollment in this study since this is one of the inclusion criteria. The mean number of the fungus cell in the AMB + FLU group was higher than that in the AMB group, but there were no significant difference (P = 0.24). All of them were confirmed the diagnosis of cryptococcal meningitis by positive CSF cultures. This suggests that india ink preparation is simple, highly sensitive and may be used as a screening diagnosis. Although, this test provides the advantages of rapid diagnosis, it should be confirmed with CSF culture. A positive CSF culture is the gold standard to definitive diagnostic test for CM.[17]

Extrameningeal examination were performed in some patients whose clinical features were indicated. There were only 7 patients who had the extrameningeal culture positive. Three patients had sputum cultures positive while lymph node, skin lesions, duodenal biopsy, and pus from ears were found cultures positive in one patient for each site.

The serum cryptococcal antigen test that has a sensitivity of 94% to 100% in HIV-infected patients with CM is not performed in this study since the changes in serum cryptococcal antigen during acute or maintenance therapy do not appear to correlate with the outcome. Thus, it could not be used to monitoring the outcome and confirm the successful of therapy. Therefore, the test should be used limited to as a screening test.[18]

2. Treatment and outcomes

Cryptococcal meningitis is the most common life-threatening opportunistic fungal disease in patients with AIDS and usually fatal if untreated. The optimum therapy for cryptococcal meningitis in patients with AIDS remains unresolved. Traditional therapy for cryptococcal meningitis in patients with AIDS was amphotericin B with or without flucytosine and has been evaluated to result in overall rate of sterilization of the CSF of 40-60% and short term mortality of 10-40%.[5,11,25,42] The addition of flucytosine to amphotericin B do not reduce mortality or shorten the duration of therapy. The toxicities associated with the two agents are the major limitations for prolonged use of the combination. The search for more efficacious and less toxic agents is continue. The oral triazoles, especially fluconazole, have increased the option for treatment of this disease. At present, the optimum therapy in patients with AIDS associated cryptococcal meningits is still controversial. New strategies and novel approaches in managing cryptococcal meningitis in patients with AIDS continue to be developed. In this study, we designed a randomized clinical trial to compare the efficacy and safety of high dose amphotericin B with or without fluconazole for treatment of cryptococcal meningitis in patients with AIDS.

As shown in Table 5, all patients received amphotericin B (0.7 mg/kg/d) in step one without a test dose of this drug since hypersensitivity reactions to amphotericin B appear to be rare upon review of the literature. Current literature does not support the recommendation to administer a test dose prior to the administration of amphotericin B because a test dose procedure potentially leads to a delay in adequate antifungal therapy that could be detrimental to a patient with a serious fungal infection such as cryptococcal meningitis. In addition, the mechanism of common adverse reactions to amphotericin B does not appear to be allergic in nature, and true allergic-like reaction is rare. New evidence suggests that the mechanism of amphotericin B in causing fever and chill is secondary to inflammatory cytokine release and alteration of neutrophil function which is not dose- or time-dependent. Nevertheless, it is prudent for nurses and pharmacists to monitor all patients for infusion-related adverse events, as well as other complications, throughout amphotericin B therapy and to collaborate with the prescriber for management recommendations when adverse events occur.[47] Majority of the patients did not receive amphotericin B premedication regimen before the first dose, only 16 patients (23.3%) received premedication later on when they were unable to tolerate to side effects (fever, shakes, and chills). Chlorpheniramine was the most commonly used premedication. The daily dose of amphotericin B was base

on each patient's weight. Most patients in this study received amphotericin B in the dosage ranging from 31 mg to 40 mg daily (41 patients, 56.9%). The mean duration of admission in both groups were 15.5 ± 5.4 days. All patients stayed in the hospital at least for 14 days because they were admininstered with amphotericin B for 14 days and had lumbar puncture at the fourteenth day of study. Many patients had a clinical outcome improvement within two weeks and were discharged to continue consolidation therapy (treatment with oral fluconazole) at home.

Table 5: Data related to amphotericin B dosage regimen of patients with AIDS-associated cryptococcal meningitis

Data	No. (%) of patients		
	AMB + FLU (n=38)	**AMB (n=34)**	**TOTAL (n=72)**
Test dose			
1. Not done	38(100.0)	34(100.0)	72(100.0)
2. Test dose	0(0)	0(0)	0(0)
No premedication	27(71.1)	29(85.3)	56(77.7)
Premedication			
1. Chlorpheniramine (CPM)	7(18.4)	5(14.7)	12(16.7)
2. Plasil	1(2.6)	0(0)	1(1.4)
3. CPM + Plasil	2(5.3)	0(0)	2(2.8)
4. Paracetamol + Plasil	1(2.6)	0(0)	1(1.4)
Dose (mg/d)			
1. < 30	14(36.9)	12(35.3)	26(36.2)
2. 31 – 40	20(52.6)	21(61.7)	41(56.9)
3. 41 - 50	4(10.5)	1(3.0)	5(6.9)
Duration of admission (day)			
Mean ± S.D.	15.3 ± 4.8	15.7 ± 6.1	15.5 ± 5.4
Range	3-30	3-30	3-30

Outcome of initial therapy at week 2

Treatment and outcomes were summerized in Figure 4. In step one, thirty-eight patients were received amphotericin B (0.7 mg/kg/d) plus fluconazole (loading dose 800 mg as single dose on the first day and 400 mg daily thereafter) and thirty-four patients received amphotericin B alone (0.7 mg/kg/d) for 2 weeks as initial therapy. Five patients from each group died before completion at week 2. The remaining 62 patients (33 patients in the AMB + FLU group and 29 patients in the AMB group) were evaluated for clinical and mycological outcome at week 2. In step two, all patients completing initial therapy received fluconazole alone of the dose of 400 mg daily or a half dose of 200 mg daily once the patient showed a first negative CSF culture and this regimen was continued for six weeks. Seven patients (3 patients in the AMB + FLU group and 4 patients in the AMB group) died and 7 patients (5 patients in the AMB + FLU group and 2 patients in the AMB group) were lost of follow-up before evaluated for clinical and mycological outcome at week 8.

Clinical and mycological outcome were evaluated separately and together as a composite outcome. Clinical outcomes were categorized into improved and not improved. Mycological outcomes were classified as conversion and persistence.

As shown in Table 6, at week 2, the mortality rate during the initial therapy for both groups were 13.2% and 14.7% (5 patients in each group) which were not significantly difference (P = 1.00). Of the 62 remaining patients who completed the course of initial therapy, most of the patients in both groups had improvement clinical outcome but the mycological outcomes were persistence. CSF cultures showed negative result in 23.7% (9 in 38 patients) of the AMB + FLU group and 32.4% (11 in 34 patients) of the AMB group. Only 27.8% (20 in 72 patients) showed improved in both clinical and mycological responses. There were no significant difference in the proportion of patient with improved clinical signs and symptoms (P = 0.64), converted to negative CSF culture (P = 0.27, one-sided test), and combined clinical and mycological responses (P = 0.27, one-sided test) between the two groups. Therefore, we were to reject the null hypothesis that amphotericin B combined with fluconazole was 30% more effective than amphotericin B alone in terms of the sterilization of CSF culture.

Figure 4: Treatment related outcomes of patients with AIDS-associated cryptococcal meningitis

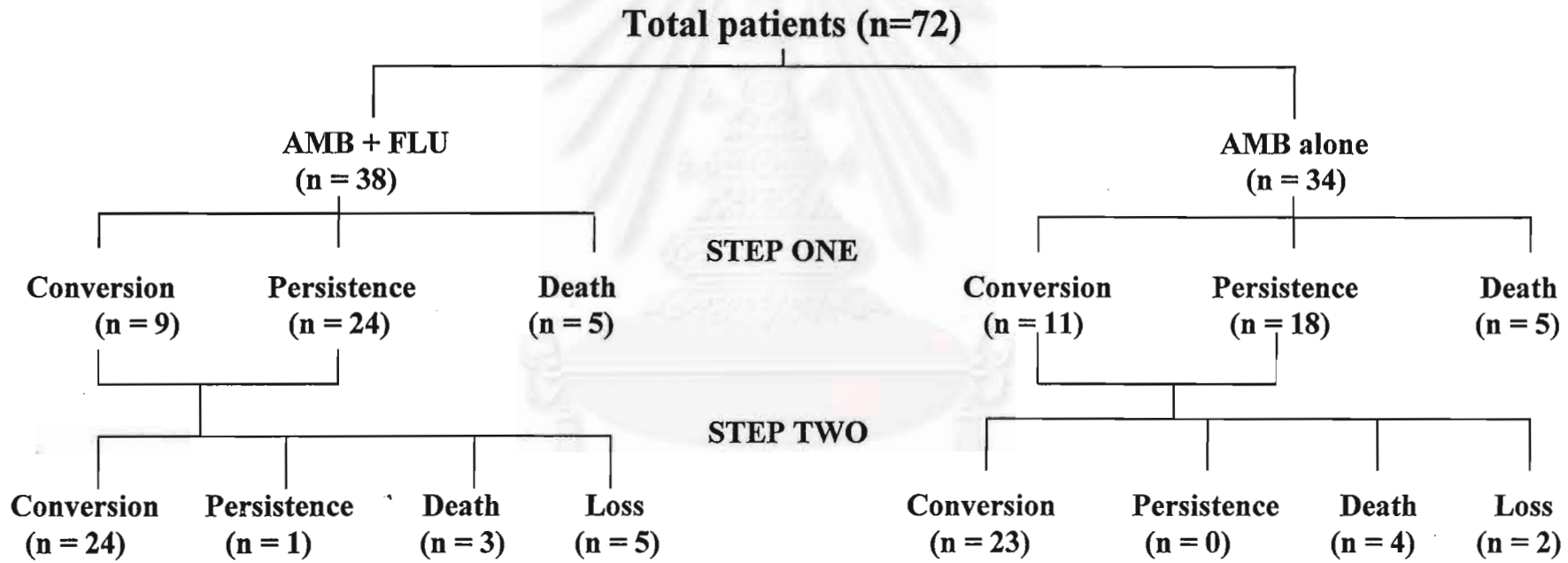


Table 6: Outcome of the initial therapy at week 2 (Step one)

Outcomes	No. (%) of patients		
	AMB + FLU (n=38)	AMB (n=34)	P-value
Survival outcome			1.00
1. Survived	33(86.8)	29(85.3)	
2. Death	5(13.2)	5(14.7)	
Clinical outcome (n=62)*			0.64
1. Improved	31(81.6)	27(79.4)	
2. Not improved	2(5.2)	2(5.9)	
Mycological outcome*			0.27
1. Conversion	9(23.7)	11(32.4)	
2. Persistence	24(63.1)	18(52.9)	

* Data were analyzed from 33 patients in AMB+FLU group and 29 patients in AMB group, 5 patients of each groups died before 2 weeks.

Table 7: The baseline CSF component values of patients with negative CSF cultures at week 2

Characteristics	Mean ± S.D.		
	AMB + FLU (n=9)	AMB (n=11)	P-value
CSF components			
1. Opening pressure (mmH_2O)	344.4 ± 141.1	292.7 ± 132.5	0.41
2. WBC (cells/mm^3)	16.9 ± 27.3	39.6 ± 60.7	0.31
3. Protein (mg/dl)	46.4 ± 13.9	49.5 ± 17.9	0.68
4. Glucose (mg/dl)	44.9 ± 14.0	45.3 ± 15.7	0.96
5. Number of fungus cell	15.7 ± 31.9	19.5 ± 31.7	0.79

In this study, the proportion of patients in both groups who had negative CSF culture were less than that obtained from the study by Charles M. Van der Horst and colleagues[11] using the same dose and experimental design as in this study (51% in AMB alone). One possible explanation for this result might be the difference in the severity of the disease. When the number of CSF negative cultures between the AMB + FLU group and the AMB group were compared, there was higher proportion of patients in the AMB group (32.4%) than the AMB + FLU group (23.7%) but this was not statistically significant different (P = 0.37). The severity of diseases of patients who received AMB + FLU appeared to be higher than patients who received AMB alone. Subgroup analysis of the CSF parameters in both groups (Table 7), patients in the AMB + FLU group had higher mean OP and lower mean CSF WBC than patients in the AMB group even though it was not statistically significant different (P = 0.41 and P = 0.31, respectively). The higher OP and lower CSF WBC have been accepted as indicators of poor prognosis.

Outcome of consolidation therapy at week 8 (Step two)

As shown in Table 8, there were 48 remaining patients (25 patients in the AMB + FLU group and 23 patients in the AMB group) for analysis of the outcomes. Seven patients (3 patients in the AMB + FLU group and 4 patients in the AMB group) died. One patient in the AMB + FLU group died from Ecthyma gangrenosum of both arms and legs with septicemia that was not related CM (confirmed by two consecutive CSF negative culture). Seven patients (5 patients in the AMB + FLU group and 2 patient in the AMB group) were lost of follow-up before completion at week 8. Three patients in the AMB + FLU group and 1 patient in AMB group had the first negative CSF cultures before they were lost of follow-up. All of the 48 remaining patients showed good clinical responses and mycological outcome except for one patient in the AMB + FLU group showed good clinical responses but persistent CSF culture. By further follow-up, this patient finally showed the CSF culture was negative at week 10.

Forty-seven patients (65.3% of 72 patients) had both clinical and mycological good responses. Subgroup analysis of the CSF parameters of both groups (Table 9) showed higher mean OP, higher protein level, higher number of the fungus cell and lower mean glucose level in the AMB + FLU group than in the AMB group. Even though all these differences were not statistically significant difference (P > 0.05) except that the mean glucose level was statistically significant difference (P = 0.01), they might cause some effects on the outcomes of the patients.

Table 8: Outcome of the consolidation therapy at week 8 (Step two)

Outcomes	No. (%) of patients		
	AMB + FLU (n=38)	AMB (n=34)	P-value
Survival outcome (n=62) *			0.70
1. Survived	30(78.9)	25(73.5)	
2. Death related CM	2(5.3)	4(11.8)	
3. Death not related CM	1(2.6)	0(0)	
Clinical outcome (n=48) **			1.00
1. Improved	25(65.8)	23(67.6)	
2. Not improved	0(0)	0(0)	
Mycological outcome**			1.00
1. Conversion	24(63.2)	23(67.6)	
2. Persistence	1(2.6)	0(0)	

* Data were analyzed from 33 patients in AMB+FLU group and 29 patients in AMB group group, 5 patients of each groups died before 2 weeks

** Data were analyzed from 25 patients in AMB+FLU group and 23 patients in AMB group 5 patients of each group died before 2 weeks, 7 patients of both groups died between two and eight weeks, and 7 patients were lost of follow-up at week 8

Table 9: The baseline CSF component values of patients with negative CSF cultures at week 8

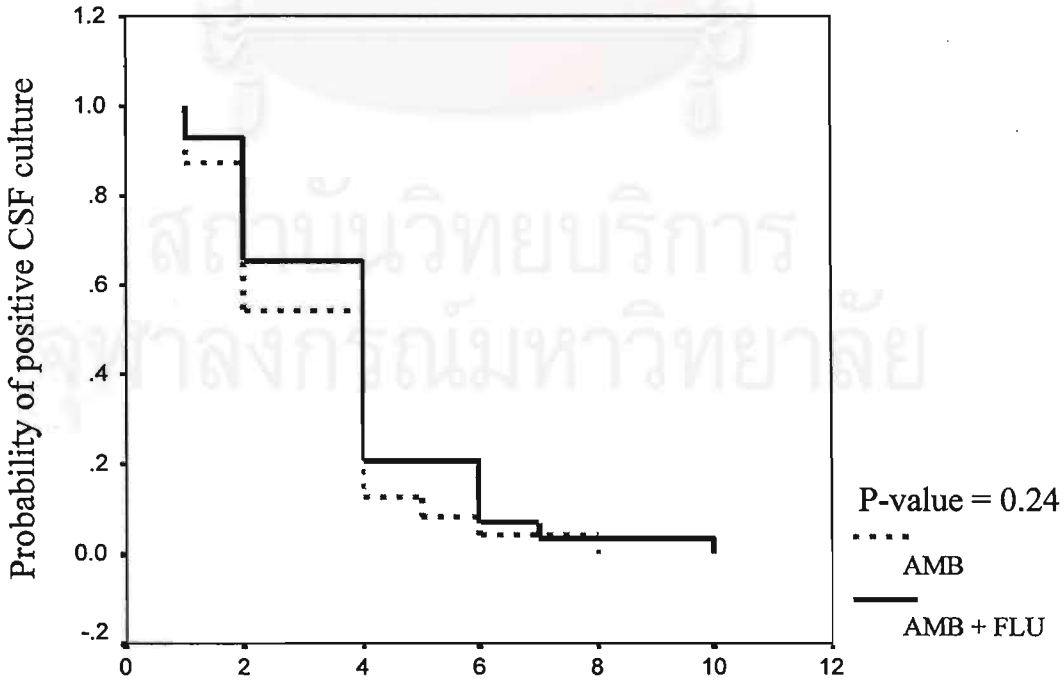
Characteristics	Mean ± S.D.		
	AMB + FLU (n=24)	AMB (n=23)	P-value
CSF components			
1. Opening pressure (mmH_2O)	367.7 ± 126.0	360.0 ± 159.5	0.86
2. WBC ($cells/mm^3$)	33.5 ± 52.7	18.1 ± 42.2	0.28
3. Protein (mg/dl)	54.8 ± 30.3	42.4 ± 15.5	0.09
4. Glucose (mg/dl)	38.2 ± 15.7	48.3 ± 10.4	0.01
5. Number of fungus cell	29.6 ± 32.4	17.9 ± 23.6	0.16

Table 10: Mycological outcomes (the first negative of CSF cultures) of patients with AIDS-associated cryptococcal meningitis

Week(s)	No. (%) of patients		
	AMB + FLU (n=38)	AMB (n=34)	TOTAL (n=72)
Conversion (n=53)*	**29(76.2)**	**24(70.4)**	**53(73.5)**
1	2(5.3)	3(8.8)	5(6.9)
2	7(18.4)	8(23.5)	15(20.8)
4	14(36.8)	10(29.4)	24(33.3)
5	0(0)	1(2.9)	1(1.4)
6	4(10.5)	1(2.9)	5(6.9)
7	1(2.6)	0(0)	1(1.4)
8	0(0)	1(2.9)	1(1.4)
10	1(2.6)	0(0)	1(1.4)
No data (n=19)	**9(23.8)**	**10(29.6)**	**19(26.5)**

* Data were analyzed from 29 patients in AMB+FLU group and 23 patients in AMB group, 16 patients of both groups died during therapy and 3 patients were lost of follow-up.

Figure 5: Kaplan-Meier estimates of the time to the first negative CSF culture



As shown in Table 10, the CSF culture was first negative at 4 weeks, in 23 patients in the AMB + FLU group (60.5%) and 21 patients in the AMB group (61.7%, P = 0.49). At 6 weeks, the CSF culture was first negative in 27 patients in the AMB + FLU group (71.0%) and 23 patients in the AMB group (67.5%, P = 1.00). At 8 weeks, the response rate determined by Kaplan-Meier estimates of the time to the first negative CSF cultures was 97.4% in the AMB + FLU group and 100% in the AMB group (Figure 5). The median times that the CSF culture was first negative were 4 weeks in both groups. There were no significant difference between the two groups in the times before the CSF cultures were negative (P = 0.24 by log-rank test). Among the patients who were treated successfully, the median times to first negative culture were also equal to 4 weeks in each groups. One patient from the AMB + FLU group and one patient from the AMB group had the first negative of CSF cultures at week 5 and 7 respectively that were out of the regular schedule of study. Because these two patients had high OP at the previous week so they were schedule to perform lumbar puncture right at instead of every two weeks.

Treatment outcomes after therapy for 8 weeks

Treatment outcomes were classified as successful and unsuccessful. As presented in Table 11, at week 8 (the end point of this study), treatment outcomes were successful in 24 of the 38 patients (63.2%) assigned to the AMB + FLU group as compared to 22 of the 34 (64.7%) assigned to the AMB alone group (P = 0.47, one-sided test). Only one patient in each group had so-called quiescent or partial response. One patient in the AMB + FLU group had the first negative of CSF culture at week 10 and other one in the AMB group had the first negative of CSF culture at week 8. Administration of amphotericin B alone, fluconazole or amphotericin B combined with flucytosine are currently recommended for the treatment of cryptococcal meningitis. The usefulness of these drugs had been limited because of their frequent toxic side effects and/or poor therapeutic effects. The results of four prospective studies reported the success rate to be about 35-50% in patients who received 0.4-0.7 mg/kg/d of amphotericin B alone.[6-8,11] However, monotherapy with low dose of amphotericin B is associated with increased mortality. The overall efficacy of amphotericin B can be improved when it is combined with flucytosine. Several studies showed the overall rate of sterilization of CSF to be 40-100% with the acute mortality rate of 10-40%.[5-11,25,48] Higher rate of sterilization of CSF were found in more than 60% patients who received the combination of high dose of amphotericin B plus flucytosine with or without triazoles antifungal agents for a period of

time which was longer than 6 weeks.[10] The major limitation of prolonged use of this combination has been their toxic effects. The development of well-tolerated and efficacious oral regimen for treatment of CM is desirable.

Fluconazole is effective in treating patients with AIDS and CM. Many studies reported the successful rate to be about 35-75% when fluconazole alone was used. The higher successful rate ranged from 50-75% was found in patients who received higher dose of fluconazole (> 400 mg daily) for longer than 8 weeks.[5-7,12-16]

In this study, the rate of success was higher than majority of those previously reported when amphotericin B (0.4-0.7 mg/kg/d) or fluconazole (≤ 400 mg/d) was used as a single agent.[5-8,12-16] However, the rate was lower than or similar to those reported when the combination of amphotericin B and flucytosine were used.[5-11,25,48] Nevertheless, the results might not be able to compare directly. There were some differences in terms of severity of the patients'CM and parameters or criteria for the evaluation of the outcomes. There are at least three possible reasons which could be used to explain why the combination of fluconazole with amphotericin B was not superior than the usage of amphotericin B alone. First, the rate of success might come to its maximum since amphotericin B was administered at a high dose (0.7 mg/kg/d). The addition of fluconazole therefore could not add any higher efficacy in the rate of sterilization of the CSF culture. Second, amphotericin B could be antagonized by fluconazole because fluconazole suppressed cytochrome P-450 activity then in turn blocks the demethylation pathway which convert 14-α-methylsterol to ergosterol and alters the primary sterol content in fungus cell membranes. Thus, fluconazole could deprive AMB of its ergosterol binding site and, hence, some of its damaging and lethal effects on fungi. However, AMB antagonism has been demonstrated only in *Aspergillus* species. Thus, true confirmation will require additional studies. Third, there were many different strains of *C. neoformans* and some strains might not response to fluconazole which was added on to amphotericin B in the first two weeks.

In this study, the mortality rate was 18.5% (7 out of 38 patients excluded one patient who died from other disease not related CM) in the AMB + FLU group while it was 26.5% (9 out of 34 patients) in the AMB group. Most of the deaths occurred during the first two weeks of therapy. Ten patients (5 patients from each groups) died during the step one of therapy and six patients (2 patients from the AMB + FLU group and 4 patients from the AMB group) died during the step two of therapy. Mortality rate for AIDS-associated acute CM reported to be 10-40% among

patients receiving amphotericin B alone as initial therpy.[5-11,25] As shown in Table 11, the mortality rates in both groups were not higher than those reported in the previous studies. Thus, both regimens used in this study showed higher success rate (approximately 60%) with low mortality rate and could therefore be used for the treatment of CM in patients with AIDS.

Of the seventeen patients who died during therapy, 7 patients had high opening pressure which was higher than 600 mmH_2O at baseline of lumbar puncture. The pathophysiologic mechanism of increased intracranial pressure (ICP) in patients with CM has not been fully elucidated. The mechanism of outflow obstruction may involve cryptococci mechanically blocking the passage of CSF across arachnoid villi, blockage by the products of inflammation, or more likely aggregration of cryptococcal polysaccharides accumulating in arachnoid villi and those subarachnoid spaces leading to lymph channels as auxillary pathways of CSF drainage. Elevated ICP in patients with AIDS-associated CM is a significant source of morbidity and mortality. The use of lumbar drainage and selective placement of lumbar peritoneal shunts in the management of elevated ICP can ameliorate the sequelae of elevated of ICP.

In this study, patient who continuously had ICP higher than 600 mmH_2O with focal neurologic deficits or mental status changes were managed by placement of intraspinal drainage. Subgroup analysis in 7 patients who had OP higher than 600 mmH_2O and were managed by placement of intraspinal drainage showed that 3 patients survived while the remaining 4 patients died due in part to the progressive of CM with other concomitant diseases and/or delayed management of the increased ICP. Raising ICP can be presented at diagnosis or can develop during therapy. Thus, patient should be routinely monitoring ICP and immediately management of raised ICP to reduce the morbidity and mortality rate caused by this disease.

Comparison of CSF parameters between the two groups during therapy were showed in Table 12. CSF parameters of most patients changed into the normal range within eight weeks of therapy. The abnormal mean of protein level decreased to normal range ($\leq$ 45 mg/dl) at week 4 in the AMB + FLU group and week 6 in the AMB group. The abnormal mean of glucose level only found in the AMB + FLU group which increased to normal range ($\geq$ 40 mg/dl) at week 2 of therapy. CSF white blood cell trend to decrease slightly in both groups. Majority of the patients in both groups had only few fungus cells at week 4 of therapy since most of them had the CSF cultures conversed to negative at the same week. The mean fungus

cells at week 8 were higher than at week 4 in the AMB + FLU group because this values were obtained from the average of fewer patients who had their first negative CSF cultures at the later weeks of therapy.

Table 11: Comparison of treatment outcomes after 8 weeks.

Outcomes	No. (%) of patients		
	AMB + FLU (n=38)	**AMB (n=34)**	**P-value**
Treatment outcomes (n=65)*			**0.47**
1. Successful**	**24(63.2)**	**22(64.7)**	
2. Unsuccessful	**9(23.7)**	**10(29.4)**	
Quiescent / partial response***	1(2.6)	1(2.9)	
Death			
- Early (≤ 2 weeks)	5(13.2)	5(14.7)	
- Late (> 2 weeks)	2(5.3)	4(11.8)	
Inevaluable†	1(2.6)	0(0)	

* Data were analyzed from 33 patients in AMB+FLU group and 32 patients in AMB group and 7 patients were lost of follow-up at week 8.

** Defined as two consecutive negative cultures from CSF samples obtained at least one week apart

*** Defined as clinical stable, progressive improvement or resolution of symptoms but persistently positive cultures from CSF or only one negative CSF cultures at the end of the 8-week treatment period

† Defined as died to another causes not related to CM during therapy

Table 12 : Comparison of CSF components between the AMB + FLU group and the AMB group

Data	Group	Lumbar puncture					
		Baseline	**Week 1**	**Week 2**	**Week 4**	**Week 6**	**Week 8**
1. OP (mm H_2O)	AMB+FLU	383.8±141.1 (n = 38)	277.7±180.5 (n = 33)	270.7±149.8 (n = 30)	326.4±142.6 (n = 25)	319.5±133.1 (n = 19)	371.7±160.3 (n = 6)
	AMB	391.5±155.6 (n = 34)	246.6±151.8 (n = 22)	263.0±146.3 (n = 28)	259.5±125.0 (n = 21)	196.9±106.9 (n = 13)	170.0±36.1 (n = 3)
2. Protein (mg/dl)	AMB+FLU	58.7±34.5 (n = 38)	73.3±41.1 (n = 35)	53.7±31.1 (n = 32)	41.9±14.2 (n = 25)	37.8±11.5 (n = 19)	36.2±7.4 (n = 6)
	AMB	46.7±19.7 (n = 34)	61.6±33.2 (n = 29)	49.6±17.1 (n = 29)	50.1±37.4 (n = 21)	44.7±19.2 (n = 13)	35.9±7.6 (n = 6)
3. Glucose (mg/dl)	AMB+FLU	37.4±17.5 (n = 38)	37.4±17.3 (n = 35)	44.1±13.8 (n = 32)	44.4±8.8 (n = 25)	47.5±9.6 (n = 19)	52.5±6.7 (n = 6)
	AMB	45.6±15.2 (n = 34)	43.5±14.6 (n = 29)	48.3±14.0 (n = 29)	46.4±7.9 (n = 21)	49.1±6.6 (n = 13)	45.0±2.0 (n = 3)
4. WBC (cells/mm^3)	AMB+FLU	25.5±44.2 (n = 38)	38.1±104.2 (n = 35)	12.3±12.3 (n = 32)	6.2±4.9 (n = 25)	6.3±6.9 (n = 19)	3.2±2.6 (n = 6)
	AMB	20.2±40.3 (n = 34)	28.8±36.8 (n = 29)	26.3±58.8 (n = 29)	9.6±14.8 (n = 21)	5.8±4.8 (n = 13)	3.0±2.6 (n = 3)
5. No. of fungus cell (cells/HPF)	AMB+FLU	36.1±34.4 (n = 38)	11.7±19.1 (n = 35)	3.2±4.2 (n = 32)	2.8±2.3 (n = 25)	3.6±3.6 (n = 19)	8.0±4.6 (n = 6)
	AMB	27.2±29.4 (n = 34)	15.3±24.1 (n = 29)	4.5±6.9 (n = 29)	1.9±1.4 (n = 21)	2.1±1.8 (n = 13)	1.7±1.5 (n = 3)

Factors related to outcomes

A number of investigators have attempted to define the clinical and laboratory characteristics associated with a poor prognosis to optimize antifungal treatment. The most important pretreatment factors associated with poor prognosis are abnormal mental status, CSF WBC count of less than 20 cells/mm^3, age lesser than 35 years, extraneural cultures positive for *C. neoformans*, and hyponatremia.[2-3,17-20,22,24]

Factors related to outcome of patients with AIDS-associated CM were shown in Table 13. Most of the patients who aged lesser than 35 years were survived which was a negative factor associated with poor prognosis. This result indicated that age of more than 35 years related with poor prognosis. Many variables, such as demographic data, clinical signs and symptoms, Glasglow Coma Scale, Karnofsky Performance Scale, and laboratory findings showed abnormal value in the death group. Neck stiffness, seizure and impaired consciousness were statistically significant appeared more in the death group ($P < 0.05$). Only some simple clinical signs and symptoms such as headache, fever, hearing loss, and lymphadenopathy were found more in the survival group than the death group. These signs and symptoms may cause by concomitant diseases not related to CM. Thus, they were not important factors associated with poor prognosis and the presence of them did not indicate good prognosis either.

The high mean of OP (445.3 ± 151.0 mmH_2O versus 369.6 ± 142.6 mmH_2O), protein level (64.4 ± 37.9 mg/dl versus 49.5 ± 25.0 mg/dl), number of fungus cell (45.9 ± 31.5 cells versus 27.6 ± 31.4 cells), low mean of CSF WBC (17.7 ± 28.8 cells/mm3 versus 24.6 ± 45.6 cells/mm^3) and glucose level (37.5 ± 22.7 mg/dl versus 42.4 ± 14.7 mg/dl) were found in the death group. However, these values were not statistically significant different between the survival and the death groups ($P > 0.05$) except for the number of fungus cell ($P = 0.04$).

In this study, abnormal mental status or impaired consciousness and number of fungus cell were found to be the significant factors associated with poor prognosis ($P = 0.02$, $P = 0.04$, respectively). Therefore, some prognosis factors identified for the patients in the West were probably not applicable to the patients in this study. Besides, these patients were different in clinical and/or laboratory characteristics and severity of the disease at the time of presentation.

Table 13: Factors related to outcomes of patients with AIDS-associated cryptococcal meningitis

Variables	No. (%) of patients		
	Survival (n=55)	**Death (n=17)**	**P-value**
Sex			0.51
1. Male	44(88.0)	12(70.6)	
2. Female	11(20.0)	5(29.4)	
Age < 35 yrs.	44(80.0)	8(47.1)	0.01
Occupation			0.74
1. Employed	38(69.1)	11(64.7)	
2. Unemployed	17(30.9)	6(35.2)	
Marital status			0.63
1. Married	39(70.9)	11(64.7)	
2. Single + Divorced	16(29.1)	6(35.3)	
Signs and symptoms			
1. Headache	54(98.2)	16(94.1)	0.42
2. Fever	37(67.3)	6(35.3)	0.02
3. Nausea / Vomiting	46(83.6)	15(88.2)	1.00
4. Neck stiffness	15(27.3)	10(58.8)	0.02
5. Cough	8(14.5)	2(11.8)	1.00
6. Dyspnea	4(7.3)	2(11.8)	0.62
7. Impaired consciousness	2(3.6)	6(35.3)	0.02
8. Seizure / Convulsion	1(1.9)	3(17.6)	0.04
9. Papilledema	4(7.4)	2(12.5)	0.61
10. Visual change	7(13.0)	4(25.0)	0.26
11. Photophobia	2(3.6)	1(6.3)	0.54
12. Hearing loss	10(18.2)	1(6.3)	0.44
13. Skin lesions	1(1.8)	0(0)	.....
14. Lymphadenopathy	10(18.2)	2(12.5)	0.72
15. Hepatomegaly	5(9.1)	2(11.8)	0.67
16. Splenomegaly	2(3.6)	1(5.9)	0.56
Glasglow Come Score			
Mean ± S.D.	14.8 ± 1.1	13.8 ± 2.1	0.10
Karnofsky Performance Score			
Mean ± S.D.	66.0 ± 11.0	51.8 ± 15.9	0.00

Table 13: Factors related to outcomes of patients with AIDS-associated cryptococcal meningitis (continue)

Variables	No. (%) of patients		
	Survival (n=55)	**Death (n=17)**	**P-value**
CSF parameters			
1. Opening pressure (mmH_2O)			
≥ 450	18(32.7)	8(47.1)	0.28
Mean ± S.D.	369.6 ± 142.6	445.3 ± 151.0	0.06
2. WBC (cells/mm^3)			
< 20	41(74.5)	14(82.4)	0.75
Mean ± S.D.	24.6 ± 45.6	17.7 ± 28.8	0.56
3. Protein (mg/dl)			
> 45	27(49.1)	8(47.1)	0.88
Mean ± S.D.	49.5 ± 25.0	64.4 ± 37.9	0.15
4. Glucose (mg/dl)			
< 40 mg/dl	19(34.5)	10(58.8)	0.07
Mean ± S.D.	42.4 ± 14.7	37.5 ± 22.7	0.42
5. Number of fungus cell (India-ink preparation)			
Mean ± S.D.	27.6 ± 31.4	45.9 ± 31.5	0.04
Extrameningeal examination			
Positive	6(10.9)	1(5.9)	1.00
Hyponatremia (Na^+ < 135 mEq/L)	18(32.7)	5(29.4)	0.80

3. Adverse events

As shown in Table 14, both treatment regimens were generally well tolerated. Chills was the most common side effect found in both groups which associated with the receiving of amphotericin B. Nausea and/or vomiting were common among patients who received amphotericin B or fluconazole. Thus, this adverse event was found more often in patients in the AMB + FLU group (55.3%) than patients in the AMB group (35.3%). Thrombophlebitis was the common adverse events that associated with intravenous of amphotericin B. There were no significant difference between the two groups in the clinical conditions of adverse events ($P = 0.17$).

Anemia and thrombocytopenia were reported to be the adverse reactions caused by both amphotericin B and fluconazole.[32-37] In this study, there were 26.4% (19 in 72 patients) with anemia and 18.1% (13 in 72 patients) with thrombocytopenia. Anemia and thrombocytopenia were found in patient who received AMB + FLU less frequently than patients who received AMB alone. Thus, the combination of fluconazole with amphotericin B did not increase anemia and thrombocytopenia. After discontinuation of amphotericin B hematocrit level, hemoglobin level, and platelet counts were gradually increase to the normal baseline which took longer than 8 weeks.

Amphotericin B may cause nephrotoxicity, as reflected by a serum creatinine level of higher than 2.0 mg/dl and the increase in blood urea nirogen (BUN) of higher than 20 mg/dl.[38] Nephrotoxicity was found in 22.2% (16 in 72 patients) of the patients. All patients in this study received high dose of amphotericin B (0.7 mg/kg/d) that may cause development of nephrotoxicity. These results agreed with the study by Melanie A. F. and collaborators[49] who reported that nephrotoxicity was significantly associated with a higher average daily dose of amphotericin B. In this study, patients received a total dose of amphotericin B ranging from 60 to 700 mg. Amphotericin B-induced nephrotoxicity was not a significant problem and the dosage were not required to reduce in these patients. All of them who developed minor sign of nephrotoxicity received supportive therapy with sodium loading and their abnormal BUN and serum creatinine level were returned to the normal baseline after discontinuation of amphotericin B for a few weeks. These results consists with one study which claimed that routine use of amphotericin B in the total dose of less than one gram would not result in significant nephrotoxicity.[50]

Hypokalemia and metabolic acidosis were common side effects found in patients who received amphotericin B. Hypokalemia in patients who received fluconazole occurred occasionally. Twenty-two patients (57.9%) in the AMB + FLU group were found hypokalemia which was more frequent than 14 patients (41.2%) found in the AMB group. This might be the additive side effect induced by fluconazole. Nevertheless, there were no significant difference between the two groups (P = 0.19). Potassium replacement therapy was administered in the patients with hypokalemia to improve such condition.

Aminotransferase and alkaline phosphatase enzymes were used to monitor the safety of treatment with fluconazole. None of the patients showed increase in aminotransferase enzymes while 5 patients (13.2%) in the AMB + FLU group and 1 patient (2.9%) in the AMB group showed an increase in alkaline phosphatase. However, there were no significant difference between the two groups (P = 0.21) and the increased values were returned to normal without other supportive therapy.

None of the patients in this study discontinued the study drugs due to adverse reactions. Fluconazole was generally well tolerated. The use of fluconazole in combination with amphotericin B did not appear to increase any serious adverse reactions.

Table 14: Adverse events during therapy

Adverse events	No. (%) of patients		
	AMB + FLU (n=38)	AMB (n=34)	P-value
Clinical conditions (n=66)*			
1. Chills	34(89.5)	29(85.3)	1.00
2. Nausea / vomiting	21(55.3)	12(35.3)	0.14
3. Thrombophlebitis	1(2.6)	4(11.8)	0.17
Abnormal laboratory (n=65)**			
1. Anemia (Fall in Hct > 5% or Hb >2g/dl)	9(23.7)	10(29.4)	0.50
2. Thrombocytopenia (Plt < 150,000 mm^3)	4(10.5)	9(26.5)	0.17
3. Increased in BUN (BUN > 20 mg/dl)	17(44.7)	19(55.9)	0.23
4. Increased in Serum Cr (SCr > 2.0 mg/dl)	10(26.3)	6(17.6)	0.42
5. Hypokalemia (K^+ < 3.5 mEq/L)	22(57.9)	14(41.2)	0.19
6. Metabolic acidosis (HCO_3^- < 22 mEq/L)	14(36.8)	15(44.1)	0.42
7. Increased in aminotransferase (> 5 times ULN)	0(0)	0(0)	.....
8. Increased in alkaline phosphatase (> 5 times ULN)	5(13.2)	1(2.9)	0.21

* Data were analyzed from 36 patients in AMB+FLU group and 30 patients in AMB group, 6 patients had no data for evaluation.

** Data were analyzed from 36 patients in AMB+FLU group and 29 patients in AMB group, 7 patients had no data for evaluation.

CHAPTER V

CONCLUSION

1. Study population

Of the 72 patients enrolled in this study, thirty-eight patients were randomly assigned to receive amphotericin B plus fluconazole (AMB + FLU) and 34 patients to receive amphotericin B alone (AMB).

Demographic data

Of the 72 patients with AIDS-associated cryptococcal meningitis, 56 patients (77.8%) were male. Most of the patients (41.7%) were between 20 and 29 years of age. The mean age was 32.5 ± 8.5 years (mean ± S.D.), with a range of 21-62 years. The three top occupations of the patients were employee (33.4%), unemployed (31.9%), and government officer (13.9%), respectively. Fifty patients (69.4%) were married. The main risk factors of HIV-infection were sexual contact as reported by 69 patients (95.8%).

Clinical characteristics

The most common presenting symptoms were headache (97.2%), nausea/vomiting (84.7%), and fever (59.7%). Neck Stiffness and impaired consciousness were documented in 25 patients (34.7%) and 11 patients (15.3%), respectively. Almost all of the clinical signs and symptoms were not significantly different between the AMB and the AMB + FLU groups ($P > 0.05$) except for fever, visual change and hearing loss which were found more often in the AMB + FLU group as compared to the AMB group and were significantly different ($P < 0.05$). Pulmonary cryptococcosis were confirmed by sputum culture positive with *C. neoformans* in 3 patients which only one patient showed skin lesions with *C. neoformans*.

The mean Glasglow Coma Scale (GCS) and Karnofsky Performance Scale (KPS) were 14.5 ± 1.5 and 62.4 ± 13.6, respectively. Most patients were not coma and required only occasional assistance. Most of the time, they were able to care for most of their own needs. Most patients were classified as mild to moderate in their diseases. The mean time of symptoms before admission was 12.3 ± 11.2 days (range, 1-60 days).

Concomitant diseases

Approximately half of the patients had concomitant diseases. About 75% of patients suffered from dual infections in addition to cryptococcal meningitis. Oral candidiasis (22.2%), herpes simplex (5.6%), and tuberculosis (4.1%) were the three most common coexisting opportunistic infections associated with cryptococcal meningits.

Laboratory findings

Low hematocrit level which was a common finding in patients with AIDS were found in both groups. The mean hematocrit level was 31.0 ± 6.5 percentage.

Sixty-six patients (91.7%) had CSF opening pressure (OP) higher than 200 mmH_2O. The mean OP averaged from both groups was 387.4 ± 147.1 mmH_2O. Of the 15 patients who had OP more than 600 mmH_2O, 7 patients died during therapy.

Analysis of biochemical CSF components showed that most patients (55 patients, 76.4%) had white blood cell (WBC) less than 20 cells/mm^3. Abnormal protein level and glucose level were found more often in the AMB + FLU group than the AMB group. There was no significant difference in abnormal protein level but abnormal glucose level was statistically significant difference between the two groups (P = 0.08 and P = 0.04, respectively). All of the patients had positive india ink and CSF culture.

Only 7 patients were found to have extrameningeal culture positive. Most often they were found from the sputum.

2. Treatment and outcomes

For the first 2 weeks, all of the patients received high dose of amphotericin B (0.7 mg/kg/d) without test dose and premedication before the first dose. Only 16 patients (23.3%) took the premedication later on when they were unable to tolerate the side effects of amphotericin B. Most patients in this study received amphotericin B dose ranging from 31 mg to 40 mg daily and the mean time of hospitalization average from both groups were 15.5 ± 5.4 days.

Outcome of initial therapy at week 2 (Step one)

Of the 72 patients, 5 patients from each group died before completion at week 2. The overall mortality rate during the initial therapy averaged from both groups was 13.9%. The remaining 62 patients (33 patients in the AMB +FLU group and 29 patients in the AMB group) were monitor further to evaluate outcomes. Most of them in either groups had clinical outcome improvement but a mycological outcome were persistence. CSF cultures showed negative results in 23.7% (9 in 38 patients) of the AMB + FLU group and 32.4% (11 in 34 patients) of the AMB group. There were no statistically significant difference in the proportion of patient with improved clinical signs and symptoms (P = 0.64), and the conversion of CSF culture from positive to negative (P = 0.27, one-sided test).

Outcome of consolidation therapy at week 8 (Step two)

Seven patients (3 patients in the AMB + FLU group and 4 patients in the AMB group) died and 7 patients were lost of follow-up before completion at week 8. There were 48 patients (25 patients in the AMB + FLU group and 23 patients in the AMB group) remained for analysis of outcomes. All of them received fluconazole alone either full dose (400 mg daily) or a half dose (200 mg daily) once the patient was found to have a first negative CSF culture. The dose was continued until eight weeks. Twenty-four patients in the AMB + FLU group and 23 patients in the AMB group showed both clinical and mycological good responses. Only one patient in the AMB + FLU group showed good clinical responses while the CSF culture was still positive. There were no significant difference in the proportion of patients who had good clinical and mycological responses between the two groups (P = 1.00).

The response rate determined by Kaplan-Meier estimates of the time to the first negative CSF cultures was 97.4% in the AMB + FLU group and 100% in the AMB group. The median times for the CSF culture to show first negative result were 4 weeks in both groups. There was no significant difference between the two groups in the time to the first negative CSF culture (P = 0.24 by log-rank test).

Treatment outcomes after therapy for 8 weeks

Treatment outcomes were successful in 24 of the 38 patients (63.2%) assigned to AMB + FLU as compared with 22 of the 34 (64.2%) assigned to AMB alone (P = 0.47, one-sided test). One patient in each group had so-called quiescent or partial response. Treatment with fluconazole combined with amphotericin B showed no better efficacy in terms of clinical and mycological outcome than using amphotericin B alone.

Factors related to outcome

In this study, impaired consciousness and number of fungus cell were found to be the significant factors associated with poor prognosis (P = 0.02, P = 0.04, respectively).

3. Adverse events

Chills, hypokalemia and metabolic acidosis were the most common adverse effects associated with amphotericin B in both groups. Nausea or vomiting was found more common in patients who received amphotericin B plus fluconazole.

Anemia and thrombocytopenia were reported 26.4% (19 in 72 patients) and 18.1% (13 in 72 patients) for both groups.

Amphotericin B – induced nephrotoxicity (defined as increased in serum creatinine level higher than 2.0 mg/dl and BUN higher than 20 mg/dl) was not found to cause any significant problem and none of the patients in this study required any reduction in the dosage due to this problem.

Increased in alkaline phosphatase enzymes (> 5 times ULN) was found in five patients (13.2%) in the AMB + FLU group and one patient (2.9%) in the AMB group. There were no significant difference between the two groups (P = 0.21) and the increased values were returned to normal within 8 week of therapy without other supportive therapy.

None of the patients in this study had to discontinue the study drugs due to the adverse reactions. Fluconazole was generally well tolerated. The use of fluconazole in combination with amphotericin B did not appear to increase in either the efficacy or the adverse drug reactions.

In summary, the use of high dose amphotericin B (0.7 mg/kg/d) as the initial or induction therapy, followed by consolidation therapy with oral fluconazole, is safe and effective and should be considered as the treatment of choice for AIDS-associated CM. In this study, the regimen using high dose of amphotericin B alone (0.7 mg/kg/d) for two weeks resulted in the successful rate of 64.7%. This successful rate was higher than those previous reports which indicated the successful rate to be about 35-50% in patients who received either amphotericin B (0.4-0.7 mg/kg/d) or fluconazole (≤ 400 mg/d) alone.[6-8,11] Therefore, this regimen is recommended for future therapy.

Elevated ICP in patients with AIDS-associated CM is a significant source of morbidity and mortality that can be presented at diagnosis or can develop during therapy. Thus, patients should be routinely monitor for their ICP and immediate management are required whenever raised ICP is found. Patients who are treated with amphotericin B should be monitor for their renal function, liver function, serum electrolytes and complete blood count during therapy.

From the results of this study, the addition of fluconazole during the initial two weeks did not show any increase in either efficacy in term of sterilization cerebrospinal fluid culture or adverse drug reactions. Further study using the combination of fluconazole with lower dose of amphotericin B (0.3-0.5 mg/kg/d) might be advantages if they could enhance an efficacy close to the usage of higher-dose amphotericin B while the cost and the adverse reactions caused by higher-dose of amphotericin B could be reduced.

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APPENDIX

APPENDIX A

Case Report Form

EFFICACY AND SAFETY OF FLUCONAZOLE COMBINED WITH AMPHOTERICIN B FOR TREATMENT OF CRYPTOCOCCAL MENINGITIS IN PATIENTS WITH AIDS

SUBJECT NO. ..... [ ] A
[ ] B

PATIENT'S NAME .....

HOSPITAL NUMBER .....

ADDRESS .....

.....

TEL. .....MOBILE PHONE .....

WARD .....

แบบบันทึกประวัติผู้ป่วย

Subject No. .....

Code [ ] A, [ ] B

Admission Summary Note

1. Admission Number						2. Hospital Number .....
3. Patient's Name			4. Ages (yrs.)			6. Weight (kgs) .....
			5. Sex [1] .male [2] female			7. Height (cms) .....
8. Patient's Address .... Tel. ..... Mobile phone			9. Marital Status [1] single [2] married [3] widowed [4] divorced			10. Occupation .....
11. Person to be notified Name ..... Address .... Tel ..... Mobile phone			12. Date of	D	M	Y
			Admission			
			Discharge			
						13..Length of stay in hospital (days) .....

14. Diagnoses

1) Principle disease injury or other condition for which patient was related

.....

2) Underlying cause of above

.....

3) Complications on other diagnoses

.....

4) Other diagnoses

.....

15. Clinical outcome	15.1 wk.1	15.2 wk.2	15.3 wk.4	15.4 wk.6	15.5 wk.8
[1] improved	[1]	[1]	[1]	[1]	[1]
[2] not improved	[2]	[2]	[2]	[2]	[2]
[3] death	[3]	[3]	[3]	[3]	[3]
[4] other	[4]	[4]	[4]	[4]	[4]
16. Mycological outcome	16.1 wk.1	16.2 wk.2	16.3 wk.4	16.4 wk.6	16.5 wk.8
[1] conversion	[1]	[1]	[1]	[1]	[1]
[2] persistance	[2]	[2]	[2]	[2]	[2]
[3] other	[3]	[3]	[3]	[3]	[3]

4 5 6 7		
□□ □ □□ □□□		
8 9 10		
□□□ □ □□		
12 A		
□□□□□□		
12 D	13	
□□□□□□	□□□	
15.1	16.1	
□	□	
15.2	16.2	
□	□	
15.3	16.3	
□	□	
15.4	16.4	
□	□	
15.5	16.5	
□	□	

17. Treatment outcome
- [1] successful
- [2] quiescent/partial response
- [3] progressive disease/death
- [4] toxic reaction
- [5] inevaluable

18. Type of discharge
- [1] with approval
- [2] against advice
- [3] death
- [4] by transfer
- [5] by escape
- [6] other.....

17	18
□	□

Attending physician .....

Signature

แบบบันทึกประวัติผู้ป่วย

Subject No. ..... Code [ ] A, [ ] B

Admission Summary Note (Continue)

BP .....mm Hg T .....°C Pulse .....

Chief Complaint

Present Illness

19. Clinical features

[1] fever	[1] Y	[2] N
[2] headache	[1] Y	[2] N
[3] nausea/vomiting	[1] Y	[2] N
[4] neck stiffness	[1] Y	[2] N
[5] cough	[1] Y	[2] N
[6] dyspnea	[1] Y	[2] N
[7] impaired consciousness	[1] Y	[2] N
[8] seizure/convulsion	[1] Y	[2] N
[9] papilledema	[1] Y	[2] N
[10] visual changes	[1] Y	[2] N
[11] photophobia	[1] Y	[2] N
[12] hearing loss,deafness	[1] Y	[2] N
[13] skin lesions	[1] Y	[2] N
[14] lymphadenopathy	[1] Y	[2] N
[15] hepatomegaly	[1] Y	[2] N
[16] splenomegaly	[1] Y	[2] N
[17] other	[1] Y	[2] N

20. Glasglow Coma Scale E.....M.....V.....

21. Karnosky Performance Scale .....

22. Severity of disease [1] mild to moderate
[2] severe

Past Medical History/Surgery

23. Risk [1] IVDU [2] contact partner
[3] received blood or blood products
[4] heterosexual [5] homosexual
[6] bisexual

Known HIV-infected [1] not known
[2] known; year .....

24. Diseases

24.1 OC^{+}	[1] Y	[2] N
24.2 Herpes Simplex	[1] Y	[2] N
24.3 Tuberculosis	[1] Y	[2] N
24.4 PCP	[1] Y	[2] N
24.5 other	[1] Y	[2] N

Acute and chronic Medical Problems :

Family and Social History :

History of Allergies :

Medication PTA :

19.1 ☐	19.2 ☐	19.3 ☐	19.4 ☐
19.5 ☐	19.6 ☐	19.7 ☐	19.8 ☐
19.9 ☐	19.10 ☐	19.11 ☐	19.12 ☐
19.13 ☐	19.14 ☐	19.15 ☐	19.16 ☐
19.17 ☐			

20	21	22	23
☐☐☐	☐☐	☐	☐&☐

24.1 ☐
24.2 ☐
24.3 ☐
24.4 ☐
24.5 ☐

แบบบันทึกข้อมูลผลการตรวจทางห้องปฏิบัติการ (1)

Subject No. ..... **Code [ ] A, [ ] B**

Lab Test	Normal Range Male	Normal Range Female	Date									
BLOOD EXAM												
Glucose (mg/dl)	75-115											
BUN (mg/dl)	4.7-23											
Creatinine (mg/dl)	0.9-1.5	0.7-1.3										
Sodium (mmol/L)	137-150											
Potassium (mmol/L)	3.5-5.3											
Chloride (mmol/L)	99-108											
CO2 (mmol/L)	24-32											
Calcium (mg/dl)	9-11.5											
Phosphorous (mg/dl)	2.5-4.8											
Magnesium (mg/dl)	1.6-2.3											
Total Protein (g/dl)	6.6-8.3											
Albumin (g/dl)	3.4-5.5											
Total Bilirubin (mg/dl)	0.2-1.0											
Direct Bilirubin (mg/dl)	0.05-0.3											
Alkaline Phos. (U/L)	35-110											
AST (SGOT) (U/L)	10-34	10-31										
ALT (SGPT) (U/L)	9-43	9-46										
URINE EXAM pH / Sp.Gr.												
Albumin												
Sugar												
RBC												
WBC												
Casts												
Epithelial Cells												

URINE CULTURE AND SENSITIVITY TEST

Name :	Age :	HN :	Ward :	Bed :

แบบบันทึกข้อมูลผลการตรวจทางห้องปฏิบัติการ (2)

Subject No. ..... **Code [ ] A, [ ] B**

Lab Test	Normal Range Male	Normal Range Female	Date									
HEMATOLOGICAL EXAM												
Hb (gm/dl)	12-15	11-14										
Hct (%)	38-48	35-41										
RBC Count (10^6/µl)	4.0-5.7	3.7-5.0										
WBC Count (10^3/µl)	4.5-9.0	4.5-8.0										
Differential (%)												
Neutrophil	36-70	35-75										
Lymphocytes	23-57	20-59										
Monocytes	2-7	2-7										
Eosinophil	1-5	1-5										
Basophil	0-3	0-3										
Band form	0-1	0-1										
Platelet Count (10^3/µl)	164-326	172-364										
MCV (fl)	80-97	77-97										
MCH (pg)	25-35	23-33										
MCHC (g/dl)	30-36	29-35										
CSF EXAM												
OP/ CP	< 200 mm H_2O											
WBC/ RBC	< 10 cells/ mm^3											
Lymphocytes												
PMN/ Monocytes												
Protein	< 45 mg/dl											
Glucose	> 40 mg/dl											
India ink												
Culture												
EXTRAMENINGEAL EXAM												
Blood												
Sputum												
Skin lesions												

OTHER TESTS

Name :	**Age :**	**HN :**	**Ward :**	**Bed :**

แบบบันทึกประวัติการใช้ยา

Subject NO. ..... page 5

Name ..... Age ..... HN ..... Ward ..... Bed ..... Treatment Code [1] A [2] B

Date start	Drug – strength – route of adminstration																				

แบบบันทึกข้อมูลผู้ป่วยกลับบ้าน

Subject No. ..... **Code [ ] A, [ ] B**

Discharge Summary Note

Discharge Medication :

Patient Counseling :

Follow up Date :

CBC (Baseline)

25.1 Hct □□.□□

25.2 WBC □.□□

CSF EXAM (baseline)

26.1 OP □□□

26.2 WBC □□□

26.3 Protein □□□.□□

26.4 Glucose □□□.□□

26.5 India-ink [1] positive [2] negative

26.6 Culture [1] positive [2] negative

EXTRAMENINGEAL EXAM

27.1 Blood [1] +ve [2] -ve [3] not done

27.2 Sputum [1] +ve [2] -ve [3] not done

27.3 Skin lesions [1] +ve [2] -ve [3] not done

27.4 LN [1] +ve [2] -ve [3] not done

POOR PROGNOSIS

28.1 Age < 35 yrs. [1] Y [2] N

28.2 Abnormal mental status [1] Y [2] N

28.3 High Intracranial pressure [1] Y [2] N

28.4 Positive India-ink [1] Y [2] N

28.5 Positive extramenin.culture [1] Y [2] N

28.6 Low CSF leukocytes [1] Y [2] N

28.7 Low CSF glucose [1] Y [2] N

28.8 Hyponatremia [1] Y [2] N

แบบติดตามการรักษา

Subject No. ..... **Code [ ] A, [ ] B**

Name ..... **Sex** ..... **Age** .....yrs. **HN** .....

BW ..... kg **BP** .....mm Hg **T** .....°C **Pulse** .....

Visit ..... (After treatment ..... week, week ....) **Date** ...../...../.....

Clinical features:					
1. fever	[1] Y	[2] N	10. visual changes	[1] Y	[2] N
2. headache	[1] Y	[2] N	11. photophobia	[1] Y	[2] N
3. nausea/vomiting	[1] Y	[2] N	12. hearing loss, deafness	[1] Y	[2] N
4. neck stiffness	[1] Y	[2] N	13. skin lesions	[1] Y	[2] N
5. cough	[1] Y	[2] N	14. lymphadenopathy	[1] Y	[2] N
6. dyspnea	[1] Y	[2] N	15. hepatomegaly	[1] Y	[2] N
7. impaired consciousness	[1] Y	[2] N	16. splenomegaly	[1] Y	[2] N
8. seizure/convulsion	[1] Y	[2] N	17. other	[1] Y	[2] N
9. papilledema	[1] Y	[2] N			

Glasglow Coma Scale E.....M.....V.....

Karnosky Performance Scale .....

Severity of disease [1] mild to moderate

[2] severe

PE:

LABS:

Lab Test	**Normal Range**		**Value**
	Male	**Female**	
BLOOD EXAM			
Glucose (mg/dl)	75-115		
BUN (mg/dl)	4.7-23		
Creatinine (mg/dl)	0.9-1.5	0.7-1.3	
Sodium (mmol/L)	137-150		
Potassium (mmol/L)	3.5-5.3		
Chloride (mmol/L)	99-108		
CO2 (mmol/L)	24-32		
Calcium (mg/dl)	9-11.5		
Phosphorous (mg/dl)	2.5-4.8		
Magnesium (mg/dl)	1.6-2.3		
Total Protein (g/dl)	6.6-8.3		
Albumin (g/dl)	3.4-5.5		
Total Bilirubin (mg/dl)	0.2-1.0		
Direct Bilirubin (mg/dl)	0.05-0.3		
Alkaline Phos. (U/L)	35-110		
AST (SGOT) (U/L)	10-34	10-31	
ALT (SGPT) (U/L)	9-43	9-46	

แบบติดตามการรักษา

Subject No. ..... **Code [ ] A, [ ] B**

Name ..... **Sex** ..... **Age** .....yrs. **HN** .....

Lab Test	Normal Range		Value
	Male	Female	
HEMATOLOGICAL EXAM			
Hb (gm/dl)	12-15	11-14	
Hct (%)	38-48	35-41	
RBC Count (10^6/µl)	4.0-5.7	3.7-5.0	
WBC Count (10^3/µl)	4.5-9.0	4.5-8.0	
Differential (%)			
Neutrophil	36-70	35-75	
Lymphocytes	23-57	20-59	
Monocytes	2-7	2-7	
Eosinophil	1-5	1-5	
Basophil	0-3	0-3	
Band form	0-1	0-1	
Platelet Count (10^3/µl)	164-326	172-364	
MCV (fl)	80-97	77-97	
MCH (pg)	25-35	23-33	
MCHC (g/dl)	30-36	29-35	
CSF EXAM			
OP/ CP	< 200 mm H_2O		
WBC/ RBC	< 10 cells/ mm^3		
Protein	< 45 mg/dl		
Glucose	> 40 mg/dl		
India ink			
Culture			
L/ PMN/ M			
EXTRAMENINGEAL EXAM			
Blood			
Sputum			
Skin lesions			
OTHER TESTS			

Medications for weeks:

[ ] 2 [ ] 4 [ ] 6 [ ] 8

1. Cryptococcal Meningitis Treatment

[ ] Amphotericin B 0.7 mg/kg/day + Drug A 400 mg/day (2 capsules)

[ ] Amphotericin B 0.7 mg/kg/day + Drug B 400 mg/day (2 capsules)

[ ] Fluconazole 400 mg/day [ ] Fluconazole 200 mg/day

2. Pneumocystic Carinii Prophylaxis

[ ] No [ ] Yes: Co-trimoxazole ..... X ..... pc

แบบติดตามการรักษา page 9

Subject No. ..... **Code [ ] A, [ ] B**

Name ..... **Sex** ..... **Age** .....yrs. **HN** .....

3. Tuberculosis Treatment

[ ] No [ ] Yes: Date start ...../...../.....

- Isoniazid (100) .....x.....hs
- Rifampicin (.....) .....x..... hs
- Pyrazinamide (500) .....x .....hs
- Ethambutol (400) .....x .....hs

4. Other Medications

[ ] No [ ] Yes

Drugs-strength-route of administration	Indication	Start Date	Stop Date
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10 .			

Clinical outcome:

[1] improved

[2] not improved

[3] death date ...../...../.....

Reasons .....

.....

[4] other.....

Mycological outcome:

[1] conversion

[2] persistence

[3] other .....

Side effects: [ ] No

[ ] Yes, please fill in the form "Adverse drug reaction report form"

Follow up Date: **Physicians:**

APPENDIX B

Clinical Assessment of severity of Cryptococcal meningitis

Clinical features	Grade	
	Mild to Moderate	Severe
1. Level of consciousness (Glasglow coma scale)	lethargy, obtundation (10-15)	stupor,coma (<10)
2. Loss of consciousness	absent	present
3. Cranial nerve palsy 3.1 papilledema	absent	present
3.2 loss of vision	absent	present
3.3 hearing loss, deafness	absent	present
4. Cutaneous Cryptococcosis (Skin lesions)	absent	present
5. Visceral Cryptococcosis 5.1 Lymphadenopathy	absent	present
5.2 Hepatomegaly	absent	present
5.3 Splenomegaly	absent	present
6. CSF opening pressure (mmHg)	200-450	> 450

APPENDIX C

Glasgow Coma Scale

Eyes open:	Spontaneously	4
	To verbal command	3
	To pain	2
	No response	1
Verbal responses:	Oriented & conversant	5
	Disoriented & conversant	4
	Inappropriate words	3
	Incomprehensible sounds	2
	No response	1
Motor response:	Obey verbal command	6
	To painful stimulus	
	Localized painful stimulus	5
	Flexion or withdrawal	4
	Abnormal flexion (decorticate rigidity)	3
	Extension (decerebrate rigidity)	2
	No response	1

APPENDIX D

Karnofsky Performance Scale

Able to carry on normal activity, no special care is needed	100	Normal, no complaints, no evidence of disease
	90	Able to carry on normal activity, minor signs or symptoms of disease
	80	Normal activity with effort, some signs or symptoms of disease
Unable to work, able to live at home and care for most personal needs, a varying amount of assistance is needed	70	Cares for self, unable to do normal activity or to do active work
	60	Requires occasional assistance but is able to care for most of his needs
	50	Requires considerable assistance and frequent medical care
Unable to care for self, requires equivalent of institutional or hospital care, disease may be progressing rapidly	40	Disabled, requires special care and hospital assistance
	30	Severely disabled, hospitalization is indicated although death not imminent
	20	Very sick, hospitalization is necessary, active supportive treatment is necessary
	10	Moribund, fatal processes progressing rapidly
	0	Dead

VITA

Mr. Chankig Puttilerpong was born on the ninth of March in 1975 at Hua Chiew Hospital, Bangkok. He graduated with Bachelor degree in Pharmaceutical Sciences (first class honors) in 1996 from Faculty of Pharmaceutical Sciences, Chulalongkorn University. His current position is a phamacist in Department of Pharmacy, Prachuapkhirikhan Hospital, Prachuapkhirikhan, Thailand.