อันตรกิริยาของไรแฟมปิซินต่อฟลูโคนาโซลในผู้ป่วยเอดส์ : เภสัชจลนศาสตร์และสังเกตการทางคลินิก

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DRUG INTERACTION OF RIFAMPICIN ON FLUCONAZOLE IN AIDS PATIENTS: PHARMACOKINETICS AND CLINICAL OBSERVATIONS

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for the Degree of Master of Science in Pharmacy
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Thesis Advisor	Associate Professor Duangchit Panomvana Na Ayudhya, Ph.D.
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ยาฟลูโคนาโซลซึ่งใช้เพื่อการรักษาโรคเยื่อหุ้มสมองอักเสบจากเชื้อราคริบโตคอกคัส อาจถูกใช้ร่วมกับไรแฟมปิซินในผู้ป่วยเอดส์ที่เป็นวัณโรคร่วมด้วย เนื่องจากอันตรกิริยาระหว่างยาคู่ดังกล่าวซึ่งอาจส่งผลสำคัญต่อการให้การรักษาผู้ป่วย การศึกษานี้มีวัตถุประสงค์เพื่อ ศึกษาอันตรกิริยาของไรแฟมปิซินต่อเภสัชจลนศาสตร์ของฟลูโคนาโซล ตลอดจนผลลัพธ์ทางคลินิก โดยเปรียบเทียบค่าเภสัชจลนศาสตร์ระหว่างผู้ป่วยที่ใช้ฟลูโคนาโซลเพียงอย่างเดียว กับผู้ป่วยที่ได้รับไรแฟมปิซินร่วมกับฟลูโคนาโซล ผลลัพธ์ทางคลินิกโดยเปรียบเทียบระยะเวลาของการเปลี่ยนแปลงผลการเพาะเชื้อ คลิปโตคอกคัส นีโอฟอร์แมนส์ ในน้ำไขสันหลังจากบวกเป็นลบ การศึกษานี้ทำในในผู้ป่วยที่ติดเชื้อราคลิบโตคอกคัสในสมอง ทั้งหมด 40 ราย ที่โรงพยาบาลบำราศนราดูร โดยแบ่งผู้ป่วยออกเป็นสองกลุ่ม กลุ่มละ 20 รายเท่าๆกัน

ผู้ป่วยส่วนใหญ่เป็นเพศชาย (ร้อยละ 70) โดยมีอายุอยู่ในระหว่างช่วง 21-48 ปี โดยใน 2 สัปดาห์แรกของการรักษาผู้ป่วยทั้งหมดจะได้รับยาแอมโฟเทอราซินบีในขนาด 0.7 มิลลิกรัม/กิโลกรัม/วัน หลังจากนั้นผู้ป่วยทั้งหมดจะได้รับยาฟลูโคนาโซล 400 มิลลิกรัม/วัน ผู้ป่วย 12 รายแรกของแต่ละกลุ่มจะถูกเก็บตัวอย่างเลือด ทั้งหมด 3 ระยะ (ระยะที่ 1: ในวันที่ 8 ของการใช้ยาฟลูโคนาโซล 400 มิลลิกรัม/วัน, ระยะที่ 2 ในวันที่ 15 ของการใช้ยาฟลูโคนาโซล 400 มิลลิกรัม/วัน และ ระยะที่ 3 ในวันที่ 8 ของการใช้ยาฟลูโคนาโซล 200 มิลลิกรัม/วัน) ระดับความเข้มข้นของฟลูโคนาโซลในเลือดที่ทำการวิเคราะห์ได้จะถูกนำมาวิเคราะห์ด้วยโปรแกรม RSTRIP เพื่อหาค่าทางเภสัชจลนศาสตร์ของยาฟลูโคนาโซล ผลการศึกษาพบว่า การได้รับไรแฟมปิซินร่วมกับฟลูโคนาโซล ทำให้ค่าทางเภสัชจลนศาสตร์ต่างๆ ของฟลูโคนาโซลเปลี่ยนแปลงไปอย่างมีนัยสำคัญ (P<0.05) คือ ค่าคงที่ของการขจัดยา (Ke) เพิ่มขึ้นร้อยละ 39.8 ค่าครึ่งชีวิตของยา (half-life) ลดลง ร้อยละ 28.19 ค่าพื้นที่ใต้กราฟระหว่างความเข้มข้นของยากับเวลาจากเวลา 0-24 ชั่วโมง (AUC_{0-24}) ลดลงร้อยละ 22.48 ค่าความเข้มข้นสูงสุด (Cmax) ลดลงร้อยละ 17.41 และค่าการขจัดยา (Clearance) เพิ่มขึ้นร้อยละ 29.99 เมื่อเปรียบเทียบค่าคงที่ของการขจัดยา (Ke) ในระยะที่ 1 2 และ 3 ไม่พบความแตกต่างกันอย่างมีนัยสำคัญทางสถิติในผู้ป่วยกลุ่มเดียวกัน แสดงว่าระดับของอันตรกิริยาไม่เปลี่ยนแปลงไปกับเวลาและขนาดของยาฟลูโคนาโซล การศึกษานี้ไม่พบความแตกต่างของการเปลี่ยนแปลงผลการตรวจน้ำไขสันหลังในผู้ป่วยทั้งสองกลุ่ม ความผิดปกติของโปรตีนและกลูโคสในน้ำไขสันหลังจะกลับสู่ภาวะปกติโดยเฉลี่ยภายใน 3 สัปดาห์ของการให้การรักษา จำนวนเม็ดเลือดขาวและค่าความดันในน้ำไขสันหลัง จะลดลงสู่ภาวะปกติทั้งสองกลุ่ม จากการเพาะเชื้อไม่พบเชื้อราคริบโตคอกคัสนีโอฟอร์แมนส์ ในน้ำไขสันหลังภายใน 6 สัปดาห์ของการรักษา ไม่พบความแตกต่างของระยะเวลาของการเพาะไม่พบเชื้อราในน้ำไขสันหลังในผู้ป่วยทั้งสองกลุ่ม (P=0.792) ไม่พบความสัมพันธ์ระหว่างค่าทางเภสัชทางจลนศาสตร์กับระยะเวลาของการไม่พบเชื้อราในน้ำไขสันหลัง ทั้งนี้อาจจะเป็นผลมาจากการที่ผู้ป่วยทุกรายได้รับยาแอมโฟเทอราซินบีในสองสัปดาห์แรกของการรักษา และระดับยาฟลูโคนาโซลในผู้ป่วยทั้งสองกลุ่มในช่วงที่ได้รับยาในขนาด 400 มิลลิกรัม/วัน อยู่ในช่วงที่สูงกว่าค่าความเข้มข้นต่ำสุดที่สามารถยับยั้งเชื้อ (MIC) ทำให้ผลการรักษาการติดเชื้อราคริปโตคอกคัส ในสมอง ไม่แตกต่างกันในผู้ป่วยทั้งสองกลุ่ม อย่างไรก็ตามเนื่องจากระดับฟลูโคนาโซลหลังจากขนาดยาถูกปรับลดลงเป็น 200 มิลลิกรัม/วัน ในผู้ป่วยที่ได้รับไรแฟมปิซินร่วมกับฟลูโคนาโซลต่ำกว่าค่าความเข้มข้นต่ำสุดที่สมารถยับยั้งเชื้อ (MIC) ดังนั้นจึงควรมีการติดตามศึกษาผลในระยะยาวต่อไปเพื่อศึกษาถึงอัตราการกลับเป็นซ้ำของโรค จึงจะได้ข้อสรุปว่าอันตรกิริยาของไรแฟมปิซินที่มีผลทำให้การขจัดยาฟลูโคนาโซลเพิ่มขึ้นถึงประมาณร้อยละ 30 นี้ ส่งผลกระทบทางคลินิกหรือไม่

ภาควิชา เภสัชกรรม ลายมือชื่อนิสิต .....

สาขาวิชา เภสัชกรรม ลายมือชื่ออาจารย์ที่ปรึกษา .....

ปีการศึกษา 2544 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

4276567433 : MAJOR HOSPITAL AND CLINICAL PHARMACY
KEY WORD: FLUCONAZOLE / RIFAMPICIN / INERACTION /PHARMACOKINETICS
NAWARAT THANOMPUANGSEREE : THESIS TITLE. (DRUG INTERACTION OF RIFAMPICIN ON FLUCONAZOLE IN AIDS PATIENTS: PHARMACOKINETICS AND CLINICAL OBSERVATIONS) THESIS ADVISOR: ASSOC. PROF. DUANGCHIT PANOMVANA NA AYUDHYA, Ph.D. THESIS COADVISOR : SOMSIT TANSUPHASWADIKUL, M.D., 84 pp. ISBN 947-03-0554-7.

Rifampicin and fluconazole may be co-administered in a number of clinical situations of opportunistic infection treatment in AIDS patients and the magnitude of the interaction between these two drugs may have a significant impact on the therapeutic outcome of patients. The purposes of this study were to study the effect of rifampicin on the pharmacokinetics of fluconazole along with some of its clinical outcome by compared the pharmacokinetic parameters between a group of patients who received only fluconazole and a group of patients who co-administered fluconazole with rifampicin. The effects of interaction on clinical outcome were studied by observations on the difference in time to negative culture for *Cryptococcus neoformans* in CSF between two groups. The study was carried in forty cryptococcal meningitis patients with AIDS, at Bamrajnaradura Hospital, divided in equal number of twenty in each groups.

Most of the patients were male (70%) ranging in aged from 21-48 years. For the first 2 weeks, all of patients were received amphotericin B (0.7 mg/kg/day), then fluconazole 400 mg/day were given to all patients. Blood sample from the first twelve patients enrolled in each group were collected in 3 periods (period I: on the day 8 of fluconazole 400 mg/day, period II on the day 15, period II on the day 8 of fluconazole 200 mg/day). Pharmacokinetic parameters were generated from fluconazole concentrations RSTRIP program. This study found that concomitant administration of rifampicin with fluconazole resulted in significantly changed in the pharmacokinetic parameters of fluconazole. These changes included 39.08 % increase in Ke, 28.19 % decrease in half-life, 22.48 % decrease in $AUC_{(0\text{-}24)}$, 17.41 % decrease the maximum concentration (Cmax) and 29.99 increase in clearance ($P<0.05$). The mean Ke derived from concentrations obtained in period I, II and III were not significantly different ($P>0.05$ by Repeated-Measures Analysis of Variance) indicated that the extent of interaction was complete and stable, did not change with time and doses of fluconazole. There were no significant differences in CSF components between the two groups. The abnormal mean protein and mean glucose changed to normal range within week 3 of the therapy. CSF white blood cell count and OP slowly decrease to normal ranges in both groups. CSF culture of all patients became negative within 6 weeks. There were no significant differences in the conversion rates of CSF culture between the two groups ($P = 0.792$). Comparison of fluconazole pharmacokinetic parameters between groups of patients who had different conversion rates (3 weeks, 4 weeks and 6 weeks) showed no significant differences. Since amphotericin B had been given during the first 2 weeks as an initial therapy for cryptococcal meningitis and fluconazole concentrations in the dosage of 400 mg/day were high above in MIC range, the clinical outcome turn out to be the same even though the concentrations and pharmacokinetic parameters of fluconazole changed significantly when co-administered with rifampicin. The mean serum concentrations of fluconazole in patients who received rifampicin concomitantly with fluconazole were lower than MIC, long term monitoring for recurrent rates of cryptococcal meningitis before any conclusion could be made on whether the 30% decrease in clearance of fluconazole from rifampicin will cause any significant effects on clinical outcome of fluconazole.

Department	Pharmacy	Student's signature	N. Thanompuangseree
Field of study	Pharmacy	Advisor's signature	Duangchit Panomvana
Academic year	2001	Co-advisor's signature	S. Tansuphaswadikul

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ABBREVIATION

AIDS	=	Acquired immunodeficiency symdrome
AMB	=	Amphoteraicin B
AUC	=	Area under the concentration-time curves
BUN	=	Blood urea nitrogen
CM	=	Crytococcal meningitis
C_{MAX}	=	Maximum concentration
CRAG	=	Cryptococcal antigen
CSF	=	Cerebrospinal fluid
FLU	=	Fluconazole
HIV	=	Human immunodeficiency virus
HPLC	=	High performance liquid chromatography
ICP	=	Intracranial pressure
IS	=	Internal standard
Ka	=	Absorption rate constant
Ke	=	Elimination rate constant
MRI	=	Magnetic resonance imagine
OP	=	Opening pressure
RIF	=	Rifampicin
$T_{1/2}$	=	Half life
T_{MAX}	=	Time to reach the maximum concentration
WBC	=	White blood cell

CHAPTER I

INTRODUCTION

Acquired Immune deficiency Syndromes (AIDS) is the importance public health problem of the world including Thailand. The total number of report cases of alive AIDS patients in Thailand now was higher than 700,000 and more than 300,000 have already died. Human immune deficiency virus (HIV) itself does not produce most of the morbidity and mortality associated with AIDS. Rather, opportunistic infections which caused by microorganisms are responsible for almost 90% of deaths.[1-3]

Cryptococcal meningitis (CM) is the most common life-threatening opportunistic fungal disease in patients with AIDS. Studies indicated that 5-10% of AIDS patients in western countries and up to 30% of those in sub-Saharah Africa would develop cryptococcal meningitis.[4-5]According to the report from Division of Epidemiology, Ministry of Public Health of Thailand during 1984 to 2000, there were 22,937 Thai patients with CM (16.9%).[2] And CM was the third most common opportunistic infection in AIDS patients after *Mycobacterium tuberculosis* (both pulmonary and extra-pulmonary) and *Pneumocystic carinii* pneumonia (PCP). The treatment goals of CM for AIDS patients are the control of infection, decrease in early mortality, prevention of relapse, and maintennance of patient's quality of life. Retrospective studies have reported the relapse rate to be 50-60% and shorter life expectancy for patients who did not receive chronic suppressive or maintenance therapy after termination primary treatment or initial therapy.[4-5] Consequently, all patients with AIDS associated CM require life long maintenance therapy to prevent relapse after completion of successful primary therapy. Primary standard therapy for CM in AIDS patients consist of amphotericin B IV 0.5-1 mg/kg/day for a minimum of 2 weeks with or without oral flucytosine 100-150 mg/kg/day in 4 divided doses and follow by oral fluconazole 400 mg/day for 8-10 weeks or until the cerebrospinal

(CSF) culture for *Cryptococcus neoformans* become negative, after that oral fluconazole 200 mg/day has been used as a prophylaxis.[6-8]

Fluconazole has been found to be very effective against several fungal species and widely used for treatment and prevent CM in AIDS patients. Fluconazole is a triazole derivative, anti-fungal activity relates to their inhibition of membrane sterol synthesis by fungal cytochrome P450 enzymes. Fluconazole distributes into a volume that approximates the total body water and therefore achieves good penetration into all body fluids including the CSF. Fluconazole is more than 90% absorbed from the gastrointestinal tract and is resistant to metabolism; the plasma half-life is independent of the dose, and the primary route of elimination is by excretion of the unchanged drug in urine. Despite the reduction in potential for drug-drug interactions because fluconazole has low affinity for human cytochrome P450 enzymes but fluconazole has showed clinically significant drug interactions.[9-11]

Drugs are often administered concomitantly to patients with AIDS and are often continued for the duration of their lives. Drugs that induce hepatic microsomal enzymes have been shown to accelerate the metabolism of fluconazole. Rifampicin is still one of the most valuable drugs for the treatment of tuberculosis. Occasionally, tuberculosis occurs simultaneously with cryptococcal meningitis in AIDS patients and concomitant administration of rifampicin and fluconazole may be required and the magnitude of the interaction between the two drugs may have a significant impact on the therapeutic outcome of the patient. Coker et al. reported a clinical relapse of cryptococcal meningitis in 3 patients associated with the concurrent administration of fluconazole and rifampicin[12]. Dupont and Drouhet also reported a decrease in fluconazole concentrations with the concurrent administration of these agents that was associated with a reduction in efficacy of treatment for oral candidiasis. In an attempt to determine whether rifampicin induces the metabolism of fluconazole, Apseloff et al reported a completed and open-label, placebo-controlled, parallel study in 16 healthy men. Rifampicin induced the metabolism of fluconazole as evidenced by a 23% lower in the area under the concentration time curve (AUC) and 25% faster elimination rate[13]. The other study by Lazar and Wilner reported that concomitant administration of fluconazole and rifampicin resulted in a 22% decrease in half-life and 23% decrease (P <0.002) in the AUC of fluconazole[14]. Limited data are available that

describe the magnitude of this interaction in patients receiving this medication concurrently. We have had no data to demonstrate the effect of rifampicin on fluconazole pharmacokinetics after multiple doses of fluconazole in patients and no data about the clinical outcome of fluconazole in patients receiving these medication concurrently.

Therefore, we designed a clinical trial to compare pharmacokinetics of fluconazole and the time to negative CSF culture in Cryptococcal meningitis patient with or without rifampicin in order to study the interaction of rifampicin on fluconazole in patients with AIDS.

Objectives

General Objectives

1. To study the interaction of rifampicin on fluconazole pharmacokinetics.
2. To study the effect of interaction between fluconazole and rifampicin on the time to negative culture for *Cryptococcus neoformans* in CSF

Specific Objectives

1. To find pharmacokinetic parameters of fluconazole in both groups of AIDS patients in Bamrasnaradura hospital.
   Group 1: The patients who received fluconazole 400 mg/day only
   Group 2: The patients who use fluconazole 400 mg/day along with rifampicin to treat other diseases, such as tuberculosis
2. To compare the pharmacokinetic parameters between the two groups: Half life ($t_{1/2}$), elimination rate constant (Ke), area under the concentration time curve (AUC), maximum concentration (C_{max}), time to reach the maximum concentration (t_{max}), clearance (Cl), volume of distribution (Vd).
3. To compare times to obtain negative culture for *Cryptococcus neoformans* in CSF between the two groups.
4. To study the relationship between the pharmacokinetic parameters and the time to obtain negative culture for *Cryptococcus neoformans* in CSF

The significance of the study

1. This study will provide information on the pharmacokinetic parameters of fluconazole in patients with or without rifampicin which can be used to solve the problems caused by the interaction between fluconazole and rifampicin.
2. This study will provide information on the clinical outcome after fluconazole has been used to treat cryptococcal meningitis in AIDS patients with or without rifampicin so one would know whether the interaction of rifampicin on fluconazole will cause significant effect which require serious concern clinically.

CHAPTER II

REVIEW OF LITERATURE

1. Cryptococcal meningitis

Cryptococcosis is a noncontaguous, systemic infection caused by the ubiquitous encapsulated soil yeast *Crptococcus neoformans,* which is found in soil, particularly in pigeon droppings, although disease occurs throughout the world, even in areas where pigeons are absent. Infection is acquired by inhalation of the organism. The incidence of cryptococcosis has risen dramatically in recent years, reflecting the increased numbers of immunocompromised patients, including those with malignancies, diabetes mellitus, chronic renal failure, and organ transplants, or those receiving immunosuppressive agents. The AIDS epidemic has also contributed to the increased numbers of patients; cryptococcosis is the fourth most common infectious complication of AIDS and the second most common fungal pathogen[4-5].

A. Etiology[4-5,15-17]

Cryptococcus neoformans is 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 infectious agents. 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 1month 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 principle 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. The 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 form AIDS patients; the most prevalent is serotype A.

B. Epidemiology[2, 15-17]

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 cases. *Cryptococcus neoformans 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 patient with AIDS. According to the report from the division of Epidemiology, Ministry of Public Health, 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[4-5, 8, 15-17]

Uncapsulated or partially encapsulated yeast released to the environment are inhaled and deposited in small airways. In the immunocompetent host, a grannulomatous 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 the lung often have no major defects of cell-mediated immunity. If the immune response is defective, proliferation and extra-pulmonary dissemination follow. When *C. neoformans* escapes from the lungs, the major secondary site of infection is the meningitis. 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 meningitis, 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 meninge, 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 toxin and evokes very little inflammatory response. Its main virulence factor is the capsular polysaccharide. 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 capsular production. Some AIDS patients have variants with small capsules those later produce large capsules when inoculated in to 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 disseminated disease.

D. Clinical manifestations [4-5, 8]

Cryptococcal disease is present in 7.5-10% of AIDS patients. Primary crytococcosis in humans almost always occur in the lungs, although the pulmonary focus usually produces a subclinical infection. Cough, rales, and shortness of breath that generally resolve spontaneously usually manifest in symptomatic infections. Disease may remain localized in the lungs or it may disseminate to other tissues, particularly the central nervous system (CNS), although the skin can also be affected. CNS involvement of *C. neoformans* can be found in 18-50% of AIDS patients. The course of crytococcal 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 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, visual disturbances (photophobia and blurred vision), 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 imagine (computed tomography or magnetic resonance imaging) is seen in up to 20% of patients, focal neurological signs or seizures are unusual and occur in only about 10% of patients.

E. Diagnosis [8, 4-5, 15-17]

Cryptococcal meningitis should be suspected in the HIV-infected patients who present with fever and/or headache, particularly with a CD_4 count below 100/mm^3. The conventional diagnostic approach is to obtain a brain-imaging scan to exclude mass effect and other CNS process. Once non-communicating 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 head is normal or shows cerebral atrophy presumably due to HIV-infection in 75-90% of patients. Non-enhancing 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[18-19]

Examination of CSF in patients with cryptococcal meningitis generally reveals an elevated opening pressure, CSF pleocytosis (usually lymphocytes), leukocytosis, a decrease CSF glucose, elevated CSF protein and a positive cryptococcal antigen. India ink smeared and cultured of CSF are specific for detecting cryptococcosis.

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 valves of the subarachoid 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 patients, 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. Specificity is near 100%. However, the presence of cryptococcus in the CSF may

persist during the first 2 month of treatment and dose not indicates 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 CSF CRAG test is 91-100%, with titers that ranges from positive only when undiluted to 1:64,000. Serum CRAG has sensitivity of 94-100% in HIV-infected patients with cryptococcal meningitis, with titers ranging from 1:1 to 1:1,000,000. 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 cerebrospinal fluid culture is the definitive diagnostic test for cryptococcal meningitis and main entry criteria in published studies. Being the gold standard, its sensitivity approaches, by definition, 100%.

F. Prognostic factors and therapeutic outcomes [4-5, 8, 20-23]

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*,

hyponatremia, the presence of one or more underlying diseases (including hematopoietic disorders and AIDS), and had corticosteroid or immunosuppressive therapy. In non-AIDS patients, the cryptococcal antigen titer can be followed during therapy to assess response to antifungal therapy. In AIDS patients, decreasing titers are not necessarily predictive of success, and titers rarely become negative at the completion of therapy.

G. Treatment[6-8, 20-25]

Anti-cryptococcal therapy should be started for patients with a clinical presentation, elevated serum CRAG, and CSF finding indicative of cryptococcal meningitis without awaiting culture results. The goals of therapy for cryptococcal meningitis in patients with AIDS are induce a remission and maintain a high quality of life. The standard therapeutic approach has been amphotericin B for both acute and maintenance therapy, though the introduction of azole compounds has changed the therapeutic approach for clinically stable patients. One retrospective evaluation supports the effectiveness of amphotericin B for treatment of cryptococcal meningitis in patients with AIDS and the value of long term suppressive therapy. The addition of flucytosine to the amphotericin B regimen was not found to enhance survival in this retrospective evaluation of patients with AIDS, which is in marked contrast to data on this combination in non-AIDS patients with cryptococcal meningitis. Early uncontrolled evaluation of the triazole compound, fluconazole, suggested efficacy of this drug for cryptococcal infections in AIDS patients. The combination of amphotericin B and flucytosine, however, was found superior to fluconazole in one small, randomized trial in patients with AIDS and cryptococcal meningitis. The largest controlled clinical trial of amphotericin B (mean daily dose 0.4 mg/kg) plus flucytosine at physician's discretion, versus fluconazole for cryptococcal meningitis, in 194 patients with AIDS found treatment was successful in 40% of the amphotericin B recipients and 34% of fluconazole. The death rate in the first 2 weeks of treatment was 18% in fluconazole arm and 14% in the amphotericin B arm. The death rate after 2 weeks was 4% and 6% respectively, in the fluconazole and amphotericin B groups. The median time to sterilization of CSF cultures was 42 days in amphotericin B recipients and 64 days in those who received fluconazole. These data suggest that while fluconazole is effective for treatment of cryptococcal meningitis,

amphotericin B is more effective because of its lower rates of early death and disease progression. Most patients with cryptococcal meningitis should probably receive amphotericin B in an intravenous dose of at least 0.5 mg/kg/day for minimum of 2 weeks as acute therapy. Flucytosine in doses of 100 to 150 mg/kg/day can be considered for combination with amphotericin B; serum concentrations should be monitored and the peak levels should be kept below 100 μg/ml to minimize hematological adverse reactions. A recent trial did not find that the addition of flucytosine to an amphotericin B regimen of 0.7 mg/kg/day for 2 weeks significantly improved mortality, clinical course, or CSF culture status at 2 weeks. Once the acute treatment of cryptococcal meningitis is completed, followed by consideration therapy with either oral itraconazole 400 mg/day or oral fluconazole 400 mg/day for 8 weeks or for obtain the negative CSF culture led to markedly improved outcomes in comparison to the results published earlier.

Cryptococcus prophylaxis[6-8]

After successful initial therapy, 50-60% of AIDS patients with cryptococcal meningitis relapses if no suppressive therapy is orally administered within the first 6 months. 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.

In addition to treatment strategies, investigations are underway to determine whether fungal infections can be prevented. Results of a controlled trial of fluconazole (200 mg/d) versus clotrimazole troches (10 mg five times daily) suggest that after a median follow-up of 35 months, fluconazole recipients had a significant benefit in terms of reduced rate of invasive fungal infection (primarily cryptococcosis) and esophageal candidiasis. For example, there was a total of 32 invasive fungal infections, 17 of which were cryptococcosis. Two cases occurred in fluconazole recipients, whereas 15 developed in clotrimazole recipients. The benefit of fluconazole therapy was greater for patients with < 50 CD4 cell/μL. The 2-year cumulative risk of cryptococcosis was 1.6% in the fluconazole group and 9.9% in the

clotrimazole group (P=0.02), in contrast to risks of 0.8% and 4.3%, respectively in patients with higher CD4 counts. There was, however, no survival difference between the groups. Despite fluconazole therapy, 10.6% of recipients developed proved or presumed candidiasis, raising the possibility of emergence of resistance to fluconazole. Drug-resistant candidiasis caused by *Candida albicans* and *Candida krusei* has been observed in patients infected with HIV who are receiving fluconazole. The central question of whether the benefit of fluconazole and other agents for prophylaxis of fungal infections outweighs the risks, including resistance, remains to be clearly delineated. At present, routine antifungal prophylaxis of cryptococcosis is not recommended. Fluconazole is recommended for chronic suppressive therapy of cryptococcal meningitis in AIDS patients. The AIDS Clinical trial Groups (ACTG) 26 study demonstrated that oral fluconazole 200 mg daily was superior to IV administration of amphotericin B 1 mg/kg weekly in preventing relapse. In addition, the fluconazole treated group showed a lower incidence of adverse drug reactions and bacterial infections.

Management of increased intracranial pressure [15, 20-21]

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 routinely measurement of intracranial pressure and management of rise intracranial pressure is critical components of therapy for such patients and can ameliorate the sequel of elevated intracranial pressure.

2. Drugs therapy

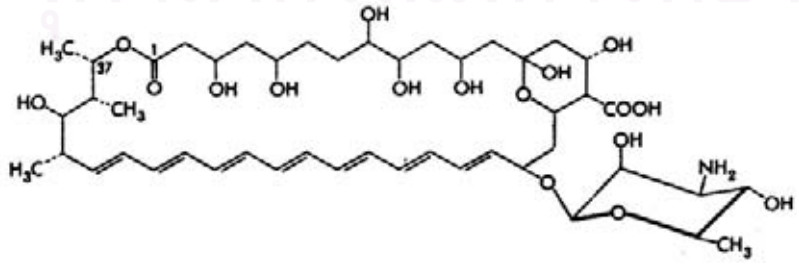
2.1 Amphotericin B[16, 24-25]

Chemistry and stability

Amphotericin B is one of a family of some 200 polyene macrolide antibiotics. Those studied to date share the characteristics of four to seven conjugated double bonds, and internal cyclic ester, poor aqueous solubility. Amphotericin B (see below for structure on figure 1) is a heptaene macrolide containing seven conjugated double bonds in trans position and 3-amino-3, 6-dideoxymannose (mycosamine) connected to the main ring by a glycosidic bond. The amphoteric behavior for which the drug is named derives from the presence of a carboxy group on the main ring and a primary amino group on mycosamine: these groups confer aqueous solubility at extremes of pH.

Amphotericin B powder for injection should be protected from moisture, light and stored at 2-8°C and must be reconstituted only with sterile water for injection because precipitation may be occur in sodium chloride or bacteriostatic agent such as benzyl alcohol. 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 it must be diluted only with 5% dextrose in water having a pH greater than 4.2.

Figure 1: Chemical structure of Amphotericin B



Antifungal Activity

Amphotericin B has useful clinical activity against *Candida spp*., *Cryptococcus neoformans, Blastomyces dermatitidis, Histoplasma capsulatum, Candida glabrata, Coccidioides immitis, Paracoccidioides braziliensis, Aspergillus spp., Penicillium marneffei*, and the agents of mucormycosis. There are inhibited by concentrations ranging from 0.03-1 mg/L *in vitro*. Some isolates of *Candida lusitaniae* have appeared to be relatively resistant to amphotericin B. Amphotericin B has limited activity against the protozoa *Leishmania braziliensis* and *Naegleria fowleri*. The drug has no antibacterial and antiviral activity.

Mechanism of action

The antifungal activity of amphotericin B depends at least in part on its binding to sterol moiety, primarily ergosterol, that is present in the membrane of sensitive fungi. By virtue of their interaction with the sterols of cell membranes, polyenes appear to form pores or channels. The result is increase in the permeability of the membrane, allowing leakage of a variety of small molecules. Additional mechanisms of action may include oxidation damage to fungal cells, at least *in vitro*.

Fungal Resistance

Mutants selected *in vitro* for nystatin or amphotericin B resistances replace ergosterol with certain precursor sterols. The rarity of significant amphotericin B resistance arising during therapy has left it unclear whether or not ergosterol-deficient mutants retain sufficient pathogenicity to survive in deep tissue. Failure of amphotericin B to penetrate the fungal cell wall of some resistant species has been suggested by the greater susceptibility of protoplasts.

Pharmacokinetics Properties

Absorption of amphotericin B from the gastrointestinal tract is negligible. Repeated daily intravenous infusions to adult of 0.5 mg/kg/day result in concentrations in plasma of about 1.0 to 1.5 μg/ml at the end of the infusion, which falls to about 0.5 to 1.0 μg/ml by 24 hours later. The drug is released from its complex with deoxycholate in the bloodstream, and the amphotericin B that remains in plasma is more than 90% bound to proteins, largely β-lipoprotein. Approximately 2 to 5% of each dose appears in the urine when patients are on daily therapy. Elimination of the drug appears to be unchanged in anephric patients and in patients receiving hemodialysis. Hepatic or biliary disease has no known effect in metabolism of the drug in human beings. At least a third of an injected dose can be recovered unchanged by methanolic extraction of tissue at autopsy; the highest concentrations are found in liver and spleen, with lesser amounts in kidney and lung. Concentrations of amphotericin B in fluids from inflamed pleura, peritoneum, synovium, and aqueous humor are approximately two thirds of trough concentrations in plasma. Little amphotericin B penetrated into cerebrospinal fluid (CSF), vitreous humor, or normal amniotic fluid. Because of extensive binding to tissues, there is a terminal phase of elimination with a half time of about 15 days.

Therapeutic Uses

The usual therapeutic dose of amphotericin B is 0.5-0.7 mg/kg/day, administered in 5% glucose over 4 hours. 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 recommend concentration for intravenous infusion is 0.1 mg/ml (1 mg/10 ml). Because patients 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/day prepared as 0.1 mg/ml infusion and delivered slowly over 2-6 hours. Depending on the patient’s cardio-renal status, dosage may be gradually increased by 5-10 mg/day up to a total dose of 0.5-0.7 mg/kg/day.

Intrathecal infusion of amphotericin B is necessary in patients with meningitis caused by *Coccidiodes*. The drug can be injected into the CSF of the lumba spine, cisterma magna, or lateral cerebral ventricle. Regardless of the site of injection, the treatment is begun with 0.05 to 0.1 mg and increased on a three-times-a-week schedule to 0.5 mg, as tolerance permits. Therapy is then continued on a twice-a-week schedule. Fever and headache are common reaction and may be decreased by intrathecal administration of 10 to 15 mg of hydrocortisone. Less common but more serious problems attend the use of intrathecal injection; the nature of the problem depends on the injection site chosen. Local injections of amphotericin B into a joint or perioneal dialysate fluid commonly produce irritation and pain. Intraocular injection following pars plana vitrectomy has been used successfully for fungal endophthalmitis, but retinal damage can occur.

Intravenous administration of amphotericin B is the treatment of choice for mucormycosis, invasive aspergillosis, extracutaneous sporotrichosis, cryptococcosis, fusariosis, alternariosis, trichosporonosis, and penicillosis. Although imidazoles or triazoles are useful in many patients with blastomycosis, histoplasmosis, coccidioidomycosis, and paracoccidioidomycosis, amphotericin B is preferred when these mycoses are rapidly progressive, occur in an immunosuppressed host, or involve the central nervous system. Amphotericin B also can be useful in selected patients with profound neutropenia and fever that is unresponsive to broad-spectrum antibacterial agents. Amphotericin B given once weekly has been used to prevent relapse in patients with AIDS who have been treated successfully for cryptococcosis or histoplasmosis.

Untoward Effects

The major acute reaction to intravenous amphotericin B is fever and chills. Sometimes hyper-apnea and respiratory stridor or modest hypotension may occur, but true bronchospasm or anaphylaxis is rare. Patients with pre-existing cardiac or pulmonary disease may tolerate the metabolic demands of the reaction poorly and, for this reason, should be given a test dose of 1 mg to assess the severity of the reaction. Although the reaction ends spontaneously in 30 to 45 minutes, meperidine may shorten it. Pretreatment with oral acetaminophen or use of intravenous hydrocortisone

hemisuccinate, 0.7 mg/kg, at the start of the infusion decrease reactions. Febrile reactions abate with subsequent infusions. Infants, children and patients receiving therapeutic doses of corticorsteroids are less prone to reactions. The mechanism of the febrile reaction is thought to be the release of interleukin-1 and tumor necrosis factor from monocytes and macrophages

Azotemia occurs in 80% of patients who receive amphotericin B for deep mycoses. Toxicity is dose-dependent and transient and is increased by concurrent therapy with other nephrotoxic agents, such as aminoglycosides or cyclosporine. Although permanent histologic damage to renal tubules occurs even during short courses, permanent functional deficits are uncommon in patients whose renal function was normal prior to treatment, unless a total dose in excess of 3 to 4 g is given (to an adult). Renal tubular acidosis and renal wasting of K^- and Mg^{2+} also may be seen during and for several weeks after therapy. Supplemental K^+ is required in a third of patients on prolong therapy. An increase in intrarenal vascular resistance is the major cause of nephrotoxicity in amphotericin B treated rats. In patients and experimental animals, loading with sodium chloride has decrease nephrotoxicity, even in the absence of water or salt deprivation. Administration of 1 liter saline intravenously on the day that amphotericin B is to be given has been recommended for adults who are able to tolerate the Na^+ load and who are not already receiving that amount in intravenous fluids.

Hypochromic, normocytic anemia is usual; the average hematocrit declined to 27% in one study. Decrease production of erythropoietin is the probable mechanism. Patients with low plasma erythropoietin may respond to administration of recombinant erythropoietin. Anemia reverses slowly following therapy. Headache, nausea, vomiting, malaise, weight loss, and phlebitis at peripheral infusion sites are common side effects. Encephalopathy has also been attributed to amphotericin B. Thrombocytopenia or mild leukopenia is observed rarely.

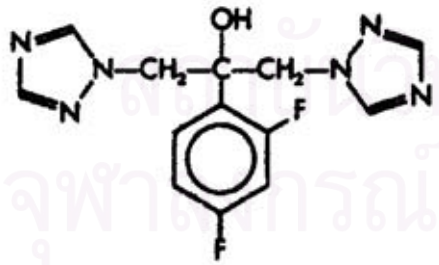
2.2 Fluconazole[11, 24-25]

Chemical 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 apparently results in increased antifungal activity and an expanded antifungal spectrum of activity. Presence of these triazole rings may contribute to fluconazole's resistance to first-pass and 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.

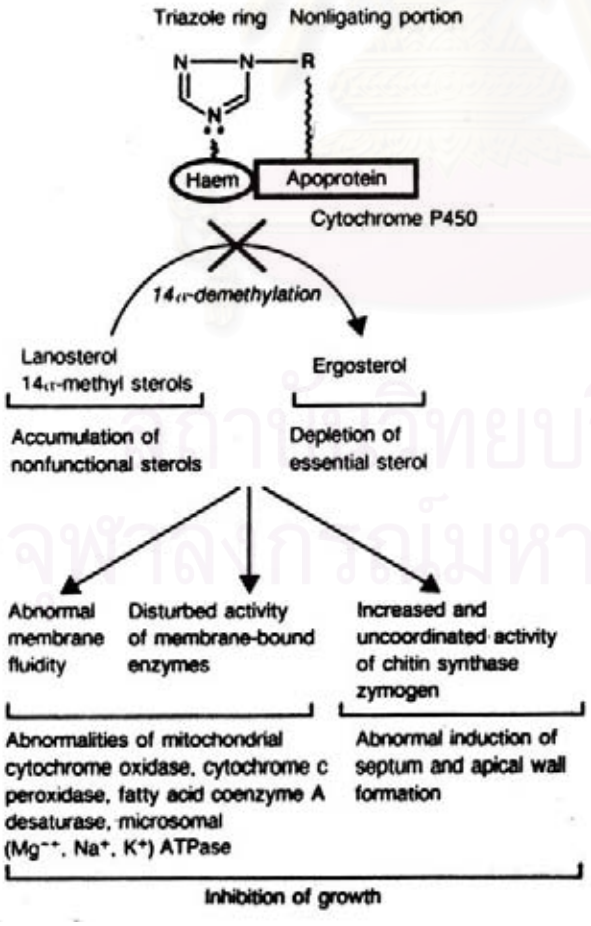
Figure 2: Chemical structure of fluconazole



Mechanism of Action

The mechanism by which azole antifungal agents interact with fungal cells to inhibit growth has been well described (figure 3). The nitrogen of the azole ring is thought to bind to the haem moiety of the fungal cytochrome P450 enzyme lanosterol 14α-demethylase, thereby halting conversion of lanosterol to ergosterol. Because ergosterol is integral to fungal membranes, a cascade of abnormalities in membrane permeability, membrane bound enzyme activity and coordination of chitin synthesis ensue. In addition, at higher concentrations azole derivatives may increase the saturation of fatty acids in the lipid bilayer. Fluconazole is a potent inhibitor of fungal lanosterol 14α-demethylase but it has much lower affinity for mammalian cytochrome P450 enzyme synthesis. The drug does not appear to have any effect on cholesterol synthesis in mammalian liver homogenates.

Figure 3: Mechainsm by azole antifungal agents[11]



Antifungal activity

Fluconazole is the most potent azole in fungal infection. Fluconazole has clinical useful activity against *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Cryptococcus neoformans*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Coccidiodes immitis*, and ringworm fungi (dermatophytes). Development of resistance to fluconazole has not been studied; however, there have been reports of cases of superinfection with *Candida* species other than *Candida albicans*, which are often inherently nonsusceptible to fluconazole. Such cases may require alternative antifungal therapy. Fluconazole dose not appears to have any useful antibacterial or antiparasitic activity.

Pharmacokinetics Properties [9, 11, 24, 27, 29, 32, 31,]

The pharmacokinetics of Fluconazole is linearly dose dependent. The summaries of fluconazole pharmacokinetics data are on table 1.

Table 1.: Summary of fluconazole pharmacokinetics data.

Parameter	value
Absolute oral bioavailability of elixir	97%
Oral bioavailability of capsule	94%
Apparent volume of distribution	0.7 L/kg
Plasma protein binding	12%
Plasma half-life	≈30
Urinary excretion	
Parent compound	64-90%
Metabolites	11%

Absorption and plasma Concentrations

Fluconazole is very well absorbed orally, with time (t_{max}) to reach peak plasma concentrations (C_{max}) within 0.5 to 6 hours after administration. Greater than 90% of an oral dose can be detected in systemic circulation. Neither food nor gastric pH modifiers (cimetidine or antacid) had a clinically significant effect on absorption in studies conducted in healthy volunteers. This was documented by a case report of favorable serum fluconazole concentrations after oral administration in a patient who had exhibited malabsorption of ketoconazole because of achlorhydria.

Protein binding

The plasma protein binding is low at 11 to 12% and fluconazole circulates as free active drug. No pharmacological, pharmacokinetic, therapeutic or toxicological consequences are to be expected on the basis of modifications in plasma protein concentration.

Distribution

Whole body autoradiography showed that fluconazole is evenly distributed to the tissues, including penetration into the gastrointestinal tract and central nervous system in mice given the drug intravenously. In humans, as in animal models, the volume of distribution approximates that of total body water (0.8-1 L/kg). In contrast to other azole antifungals, which are highly bound to plasma proteins binding of fluconazole is low (approximately 11%) and thus, most fluconazole circulates as free drug. Fluconazole is it extensive penetration into the CSF. Several studies have demonstrated that the penetration ratios of fluconazole are in range of 0.5-0.9.

Elimination Half-life

In all studies, the $t_{1/2}$ of the fluconazole was very constant, by the mean ◊ SD of 31.6 ◊ 4.9. The pharmacological consequence of this long t1/2 is that 5-7 days are needed to reach steady state.

Metabolism

Metabolism pathways for fluconazole are not qualitatively or quantitatively significant. In mice and dogs, 81 and 71% of intravenous dose, and 75 and 64% of the oral dose, respectively, were excreted in urine as unchanged fluconazole. In humans the unchanged fraction of the drug in urine is in the range of 64 to 90%. 11.4% of an administered dose was recovered in urine as metabolites over a period of 10 days in 3 healthy volunteers. Only 3 metabolites were isolated, one of which was identified as a 1, 2, 4-triazole compound. No metabolites represented more than 4% of dose. Very little metabolism occurred during the first pass through the liver after gastrointestinal absorption, thus, excellent bioavailabitity is seen for the oral drug. If hepatic function is altered, no modification to the pharmacokinetics of the drug is expected and consequently no dosage modification is necessary.

Renal Elimination

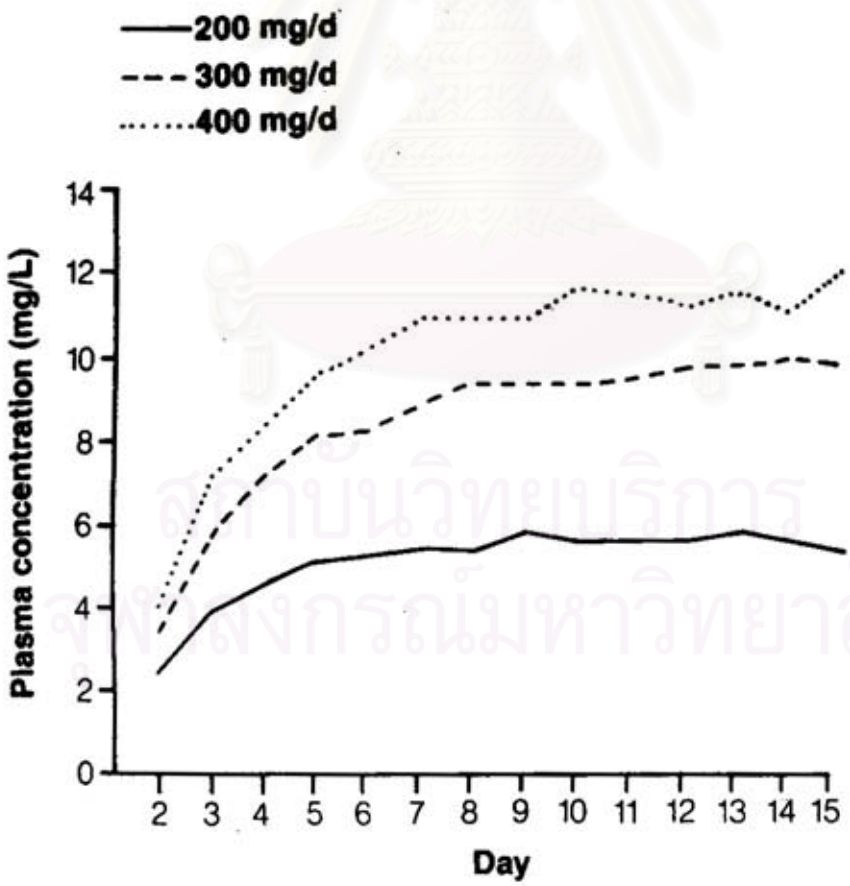
The mean ◊ SD total clearance was 19.5 ◊ 4.7 ml/min (1.17 ◊ 0.28 L/h), the mean ◊ SD renal clearance was 14.7 ◊ ml/min (0.88 ◊ 0.22 L/h) and the mean ratio of renal: plasma clearance values of 78% confirms the 73 ◊ 8% of drug eliminated unchanged by kidney. The process of renal elimination consists of 2 phenomena: high renal filtration attributable to low plasma protein binding, and extensive tubular reabsorption, which explains the long $t_{1/2}$.

Plasma Concentrations of Fluconazole

Over the range of 50 to 400 mg/day, Fluconazole plasma concentrations and area under the plasma concentration-time curve (AUC) increased in direct proportion to the dose administered. The mean peak plasma concentration (C_{max}) measured in healthy volunteers after a single 100 mg oral dose of fluconazole was 1.9 mg/L; after a single 400 mg oral dose the C_{max} was 6.7 mg/L. Plasma concentrations 15 minutes after a single 30-minute infusion of fluconazole 50 mg and 100 mg were 0.94 mg/L and 2.1 mg/L. In multiple dosing of fluconazole leads to an increase in peak plasma concentration of approximately 2.5 times, that achieved after a single dose.

In one double blind, placebo-controlled investigation, each of 60 healthy subjects was assigned to one of four treatment groups: 200 mg, 300 mg, or 400 mg of oral fluconazole or placebo given once daily. As in earlier studies, fluconazole was rapidly absorbed and plasma levels peaked between 1.2 and 2.3 hours. Peak plasma concentrations and AUC values were linearly proportional to dose. There was little inter-subject variability in the rates of elimination, which were 31, 34, and, 34 hours for 200, 300, 400 mg dosages, The values for drug accumulation (base on the ratio of day 14 to day 1 AUCs) were 2.2, 2.9 and 2.6 for the three respective dosages-values consistent with a plasma elimination half-life of ≈ 30 hours. Steady state drug levels were attained by approximately the seventh day of dosing and did not rise subsequently (Figure 4).

Figure 4: Mean trough concentrations of fluconazole in healthy volunteers receiving



the indicated doses[31]

Concentrations of Fluconazole in Other Body Fluids[31]

Except in urine, where concentrations are 10 to 20 times as great as those in plasma, concentrations in CSF, saliva, sputum, blister fluid, joint fluid, vaginal secretions, and dialysate are nearly equal to those in blood. Ratios of fluid: blood concentrations are between 0.42 and 1.36 (mean ± SD = 0.81 ± 0.21)

Therapeutic Use[9, 11]

The majority of clinical experience with fluconazole has been in patients with candidiasis or cryptococcosis. Fluconazole 50 to 100 mg daily produced rapid resolution of signs and symptoms of oropharyngeal candidiasis associated with AIDS or treatment of malignacy. 88 to 100% of patients were clinical cured and cultures became negative in 50 to 90% of patients. The recommended dosage in patients with oropharyngeal or oesophageal candidaisis is 200mg on the first day followed by 100 mg/day for minimum of 2 weeks. A single dose of 150 mg is recommended in women with acute candidiasis.

Use of fluconazole has been particularly promising in patients with AIDS related cryptococcal meningitis in whom conventional therapy with amphotericin B and flucytosine, while effective, is difficult to administer and carries considerable risk of toxicity. Fluconazole 200-400 mg daily dose cured or improved approximately 60% of patients. Or after whom conventional therapy with amphotericin B and flucytosine for 2 weeks fluconazole 400 mg/day used for consideration therapy for 8-10 weeks or for CSF cultures became negative. Fluconazole (generally 100 or 200 mg/day) has also been evaluated as maintenance therapy in patients with AIDS who had negative CSF cultures after primary treatment.

Efficacy of fluconazole

Expect for *C. krusei*, minimum inhibitory concentrations (MIC) of fluconazole are low for pathogenic yeast such as candida or cryptococcus. They are highest for dermatophytes specially *aspergillus* spp. The ranges of reported MICs were 0.4 to 0.8 mg/L or 0.125 to 0.5 mg/L, 0.2 to 3.9 mg/L for *C. albican*, higher at 0.78 to 6.25 mg/L and 0.39 to 3.13 mg/L for *C. tropicalis* and *C. parapsilosis*, respectively and > 100 mg/L for *A. fumigatus*, *A. flavus*, and *A. niger*. Thus prediction of efficacious concentrations in patients on the basis of MIC values determined *in vitro* are uncertain. Other study[43] of fluconazole susceptibility *in vitro* activity showed the MIC (90) range against 566 clinical isolates of *C. neoformans* to be 8-16 mg/L. Another study[42] reported the range of MIC of 23 strains of *C. neoformans* isolated from CSF of 16 AIDS patients with meningitis or encephalitis, to be 2-8 mg/L. One more study from a total of 28 isolates of C. neoformans from 25 AIDS patients [41] reported fluconazole MIC at which 50% and 90% of the isolates were inhibited [MIC (50) and MIC (90)], to be 4 mg/L and 16 mg/L respectively. On personal study at Bamrasnaradura hospital in Thailand[45] reported the range of MIC of 29 strains of *C. neoformans* isolated from CSF of AIDS patients to be 4-16 mg/L, MIC (50) was 8 mg/L, MIC (90) was 16 mg/L and the mean MIC was 8.55 mg/L.

Adverse Effects[24]

Fluconazole has generally been well tolerated. In clinical trials utilizing dosages between 50 and 400 mg daily, the overall incidence of adverse reactions was approximately 16% with nausea, headache, skin rash, abdominal pain, vomiting and diarrhea being reported most commonly. Only 1.5% of patients discontinued treatment due to adverse reactions and 1.3% due to laboratory abnormalities, most frequently liver function test abnormalities. Rarely, patients with AIDS have developed exfloliative skin reaction during treatment, but the role of fluconazole in this reaction is uncertain. Hepatotoxicity has also been reported rarely in patients with serious underlying disease receiving fluconazole in addition to other potentially hepatotoxic agents. The causal association of these reactions with fluconazole is uncertain but, because of their potential seriousness, patients who develop liver function abnormalities or skin rash during treatment should be monitored closely.

Drug Interactions[10, 14]

The mechanism of action of fluconazole is the inhibition of the fungal cytochrome P450-mediated C-14 demethylase, which involves the conversion of lanosterol to ergosterol leading to detertiorated cell membranes. The IC_{50} (concentration causing 50% inhibition) for *C. albicans* C-14 demethylase was 0.015 mg/L when the IC_{50} for mammalian demethylase from rat hepatic microsome was > 300 mg/L. Fluconazole appeared to have high specificity for fungal cytochrome P450 and only weak inhibiting effects on drug-metabolism cytochrome P450. On the other hand significant induction of cytochrome P450 occurred at fluconazole doses low as 10 mg/kg/day (comparable with therapeutic dose in humans) administered for 7 days. Fluconazole preferentially induced *N, N*-dimethyl aniline *N*-demethylase and *p*-nitroanisole *O*-demethylase, which are phase I enzymes and induction was dose dependent. Concentrations of uridine diphosphate glucuronosyl transferase, a phase II enzyme, were significantly lowered by fluconazole at 160 mg/kg/day. Therefore, fluconazole displays a biphasic effect (an inhibitory phase followed by a phase of induction) and clinical interactions are consequently difficult to predict. In the finding of Morita et al. in mice and humans indicated that fluconazole could be a potent inhibitor of certain cytochrome P450 isozymes mediating drug metabolism in humans.

Effect of Co-administered drugs

Rifampicin

Rifampicin is potent inducer of microsomal enzymes, there have been shown to accelerate the metabolism of fluconazole. Rifampicin and fluconazole may be coadministered in a number of clinical situations, and the magnitude of the interation between the 2 may have significant decrease fluconazole's pharmacokinetics parameters and impact on the therapeutic outcome of the patients.[10]

Apseloff et al.[13] completed an open-label, placebo-controlled, parallel study in sixteen healthy men. Subjects were randomized into two groups of 8. Each received 200 mg of oral fluconazole on day 1 and 22. Group I received 600 mg of oral

rifampicin daily on day 8 through 27, whereas group II received placebo. On the day 1 and 22, all subjects were collected blood samples at 1, 2, 4, 6, 8, 12, 18, 24, 36, 48, 72, 96, 120, and 144 hours post-dose. The study shown that, concomitant administration of fluconazole and rifampicin resulted in significant changes the pharmacokinetics of fluconazole. These changes included both 23% decrease in AUC and a 25% increase in Ke, characteristic of increased elimination of the drug had statistically different from placebo (P = 0.0018 and P = 0.0008, respectively). The mean elimination half-life before treatment with placebo approximately 32 hours; the half-life decreased to approximately 26 hours after administration of rifampicin. 19% decrease occurred in the half-life of fluconazole in this study, suggesting that patients who receive rifampicin for tuberculosis may need increased dosages of fluconazole for treatment of concomitant fungal infections.

Lazar and Wilner[14], the open-label, placebo-controlled, parallels study. A single dose of fluconazole was administered on day 1 to 18 normal healthy male vulunteers. After a washout period of 7 days, 10 of the volunteers received 600 mg of rifampicin daily and 8 received placebo for 20 days (day 8-27). On day 22, both groups received as single 200 mg dose of fluconazole 2 hours before receiving the rifampicin or placebo dose. On day 1 and 22, serum samples were collected for fluconazole determinations up to 48 hours after fluconazole dosing. The study showed that concomitant administration of fluconazole and rifampicin resulted in a 22% decrease in the half-life and a 23% decrease (P<0.002) in the AUC (extrapolated to infinity) of fluconazole from baseline values.

Tuker et al.[34] report a case in which the fluconazole area under the concentration-time curve (AUC) during combined fluconazole-rifampicin therapy was 56.1% of the fluconazole AUC with single –drug use. The patient had AIDS, along with an infection of Mycobacterium avium-Mycobacterium intracellulare. However, it was noted that the decrease in fluconazole serum levels did not appear to be as great as the comparable reductions in ketoconazole and itraconazole serum concentrations when those azole-type antifungals were given concomitantly with rifampicin in other patients.

Coker et al.[12] had reported on three AIDS patients with cryptococcal meningitis in whom clinical relapse seemed to be associated with concurrent administration of fluconazole and rifampicin. These cases showed a possible drug interaction with important clinical consequences, and they suggested doctors dhould be vigilant when these drugs are used concurrently and alert to the possibility of relapse.

Dupont and Drouhet[16] also reported a decrease in fluconazole concentrations with the concurrent administration of these agents that was associated with a reduction in the efficacy of treatment for oral candidiasis.

Nicolau et al.[35] reported the influence of rifampicin coadministration on the pharmacokinetics of fluconazole in 2 critical ill patients. The pharmacokinetics of fluconazole are reported in 5 patients in the intensive care unit (ICU), 2 of whom received rifampicin and 3 who received only fluconazole. This report found a statistically significant lowering of the AUC (52%) and a 93% higher total body clearance of fluconazole in patients treated with rifampicin. Although limited data are available describing the magnitude of the interaction between fluconazole and rifampicin in patients, This data suggest a more significant interaction than previously reported. If the concurrent administration of 2 drugs is unavoidable, the patient's clinical response to treatment should be monitored closely, as the unexpectedly large reduction in fluconazole serum concentrations may lead to poor treatment outcomes.

Jaruratanasirikul S. and Kleepkaew A.[44] are study the effect of fluconazole on rifampicin pharmacokinetics, in eleven AIDS patients received rifampicin 300 mg/day on the day 1-28 and fluconazole 400 mg/day on the day 15-28. Rifampicin pharmacokinetics was studied on day 14 and 28. There was no significant effect of fluconazole on rifampicin pharmacokinetics. These results suggest that rifampicin dosage adjustment may not be necessary when this drug is coadministered with fluconazole.

Other concomitant drugs[10]

Fluconazole significantly increases plasma levels of phenyltoin, zidovudine, rifabutin, cyclosporin, sulphonylureas, and warfarin but causes little change in the metabolism of theophylline, or oral contraceptives. Fluconazole appears less prone to elevate terfenadine levels than dose ketoconazole or itraconazole. Patients who receive more than 400 mg daily or azotemia patients who have elevated fluconazole blood levels may experience drug interactions not otherwise seen.

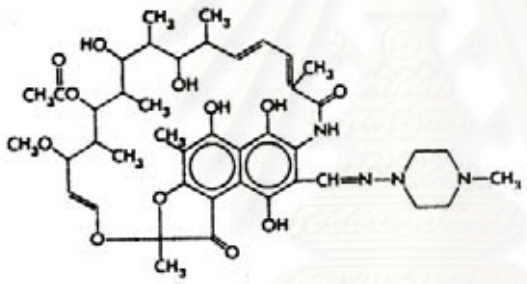
3. Rifampicin (Rifampin) [28]

The rifamycins are group of structurally similar, complex macrocyclic antibiotic produced by *Streptomyces mediterranei*; *rifampin* (RIFADIN; RIMACTANE) is a semisythetic derivative of one of theserifamycin B

Chemistry

Structure of rifampicin is in figure 5. Rifampicin is soluble in organic solvents and in water at acidic pH.

Figure 5: Chemical structure of rifampicin



Antibacterial Activity

Rifampin inhibits the growth of most gram-positive bacteria as many gram-negative microorganisms such as *Escherichia coli*, *Pseudomonas*, indole-positive and indole-negative *Proteus*, and *Klepsiella*. Rifampin is very active against *Staphylococcus aureus* and coagulase-negative staphylococci; bactericidal concentrations range from 3 to 12 ηg/ml. The drug also is highly active against *Neisseria meningitidis* and *Haemophilus influenzae*; minimal inhibitory concentrations range from 0.1 to 0.8 μg/ml.

Rifampin in concentrations of 0.005 to 0.2 μg/ml inhibits the growth of *M. tuberculosis in vitro*. Among nontuberculous mycobacteria, *M. kansasii* is inhibited by 0.25 to 1 μg/ml. The majority of strains of *M. scrofulaceum*, *M. intracelluare*, and *M. avium* are suppressed by concentrations of 4 μg/ml, but certain strains may be resistant to 16 μg/ml. *M. fortuitum* is highly resistant to the drug. Rifampin increases the *in vitro* activity of streptomycin and isoniazid, but not that of ethambutol, against *M. tuberculosis*.

Mechanism of action

Rifampin inhibits DNA-dependent RNA polymerase of mycobacteria and other microorganisms by forming a stable drug-enzyme complex, leading to suppression of initiation of chain formation (but not chain elongation) in RNA synthesis. More specifically, the β subunit of this complex enzyme is the site of action of the drug, although rifampin binds only to the holoenzyme. Nuclear RNA polymerase from a variety of eukaryotic cells does not bind rifampin, and RNA synthesis is correspondingly unaffected. While rifampin, can inhibit RNA synthesis in mammalian mitochondria, considerably higher concentrations of the drug are required than for the inhibition of the bacterial enzyme. Rifampin antibiotics also inhibit viral DNA-dependent RNA polymerases and reverse transcriptase. Rifampin is bactericidal for both intracellular and extracellular microorganisms.

Pharmacokinetics

Following absorption from the gastrointestinal tract, rifampin is eliminated rapidly in the bile, and an enterohepatic circulation ensues. During this time, the drug is progressively deacetylated, such that after 6 hours nearly all of the antibiotic in the bile is in the deacetylated from. This metabolite retains essentially full antibacterial activity. Intestinal reabsorption is reduced by deacetylation (as well as food), and metabolism thus facilitates elimination of the drug. The half life of rifampin varies from 1.5 to 5 hours and is increase in the presence of hepatic dysfunction; it may be decreased in patients receiving isoniazid concurrently who are slowinactivators of this drug. The half-life of rifampin is progressively shortened by about 40% during the

first 14 days of treatment, owing to induction of hepatic microsomal enzymes with acceleration of deacetylation of the drug. Up to 30% of a dose of the drug is excreted in urine and 60% to 65% in the feces; less than half of this may be unaltered antibiotic. Adjustment of dosage is not necessary in patients with impaired renal function.

Therapeutic Uses

Rifampicin is available alone and as a fixed dose combination with isoniazid (150 mg of isonaizid, 300 mg of rifampicin). Rifampicin and isoniazid are the most effective drugs available for treatment of tuberculosis. The dose of rifampicin for treatment to tuberculosis in adults is 600 mg, given once daily, either 1 hour before or 2 hours after a meal. Rifampicin should never be used alone for this disease because of the rapidity with which resistance may develop.

Untoward Effects

Rifampicin generally is well tolerated. When given in usual doses, fewer than 4% of patients with tuberculosis have significant adverse reactions; the most common are rash (0.8%), fever (0.5%), and nausea and vomiting (1.5%). The most notable problem is the development of jaundice. Hepatitis from rifampin rarely occurs in patients with normal hepatic function; likewise, the combination of isoniazid and rifampicin appears generally safe in such patients. However, chronic liver disease, alcoholism, and old age appear to increase the incidence of severe hepatic problems when rifampin is given alone or concurrently with isoniazid.

Because rifampicin is a potent inducer of hepatic microsomal enzymes, its administration results in a decreased half life for a number of compounds, including digitoxin, quinidine, ketoconazole, fluconazole, itraconazole, propanolol, metoprolol, clofibrate, verapamil, methadone, cyclosporine, corticosteroids, oral anticoagulants, theophylline, barbiturates, oral contraceptives, halothane, and the sulfonylureas. This effect appears about 5 to 8 days after rifampicin administration is stared and persists for 5 to 7 days after it stopped.

CHAPTER III

PATIENTS AND METHOD

The study was conducted from October 2000 to July 2001 at Bamrasnaradura Hospital, Nonthaburi, Thailand.

Patients

Study population

This study was designed as a prospective trial to compare pharmacokinetic parameters of fluconazole and the time to negative CSF culture of cryptococcal meningitis in patients with AIDS between the patients who received fluconazole alone with the patients who received rifampicin concomitant with fluconazole. 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 were admitted at the medicine ward, Bamrasnaradura hospital. Written informed consent had to be given by the patients or his legal guardian. Patients who were diagnosed cryptococcal meningitis and given a standard treatment; amphotericin B 0.7 mg/kg/day for 14 days and followed by fluconazole 400 mg/day would be enrolled in this study. Divided the patients in 2 groups; Group 1: the patients received only fluconazole 400 mg/day and Group 2: the patients also received rifampicin to treat another disease concomitant with the use of fluconazole. The criteria for enrollment were as followed:

Inclusion criteria

The patients who had all of these characteristics were enrolled in this study.

1. AIDS patients associated with cryptococcal meningitis who admitted at the medicine ward, Bamrasnaradura hospital.

2. Patients who were diagnosed cryptococcal meningitis and given a standard treatment; amphotericin B 0.7 mg/kg/day for 14 days and followed by fluconazole 400 mg/day until CSF culture for *Cryptococcus neoformans* were negative.
3. The patients consented to enroll in this study.
4. An age of 18 years or older.
5. The patients did not miss all the arrangement and took all medicine as directed.

Exclusion criteria

The patients who had either one of these characteristics were excluded from this study

1. The patients had concomitant therapy, which might change the pharmacokinetic properties of fluconazole such as carbamazepine, phenyltoin and ritonavia (except for rifampicin, which was the purpose of this study).
2. The patients had received rifampicin and stopped for less than 3 months before the starting of this study.
3. They had known allergy to polyene or azoles antifungal.
4. The patients were diagnosed from physicians to be inappropriate to enroll in this study.

Method

Study design and sample collection

Forty AIDS patients with cryptococcal meningitis (CM) who met the aforementioned criteria were participated in this study. They were divided in two groups, twenty of them received rifampicin for the treatment of tuberculosis. All patients received the standard treatment for CM: amphotericin B 0.7 mg/kg/day for 14 days followed by fluconazole 400 mg/day. The blood samples were collected from the first twelve patients enrolled in each group. The sample collections were conducted in

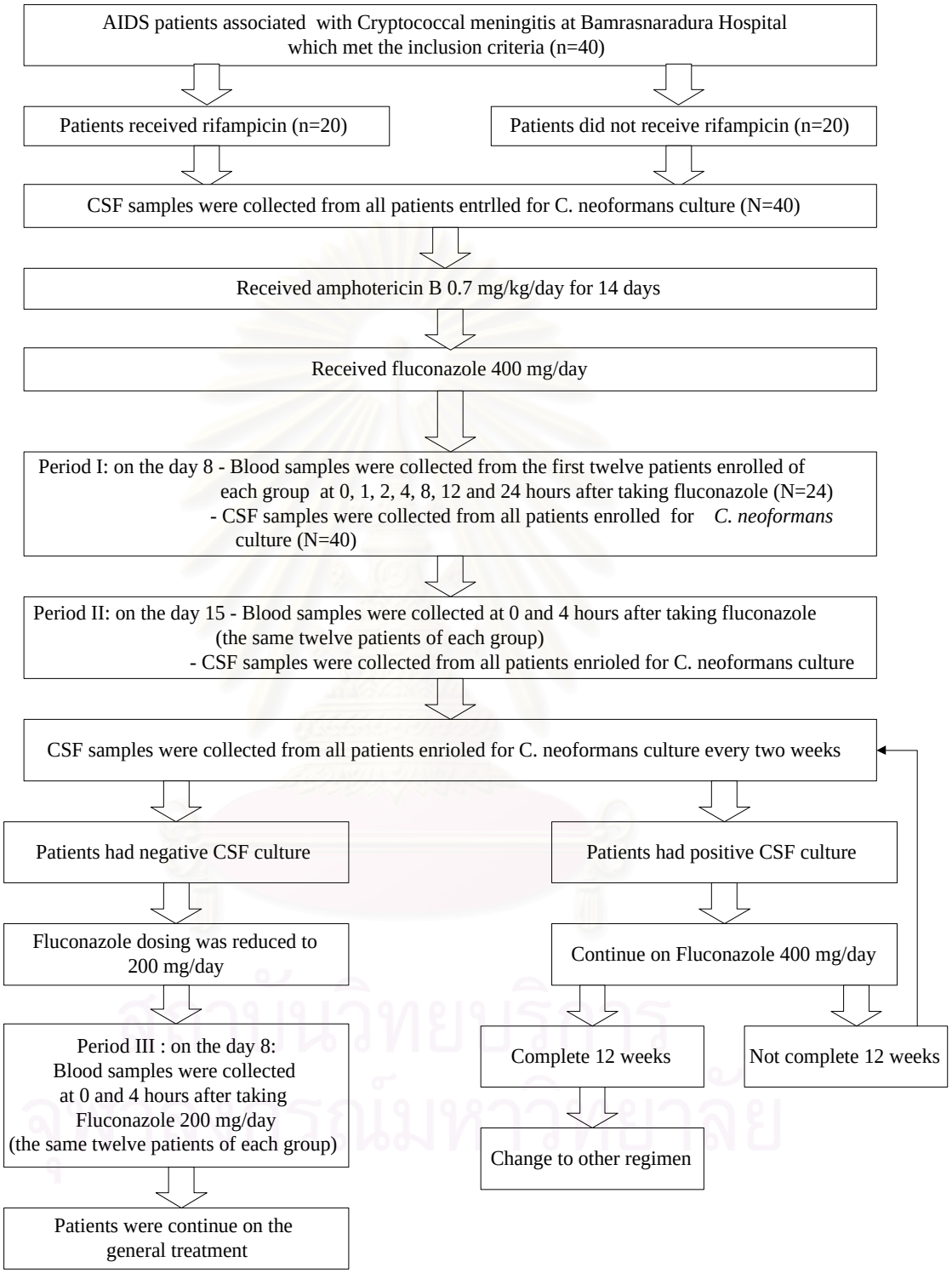
three periods (Figure 6). In period I, on the day 8 of fluconazole 400 mg/day, 5-10 ml of blood samples were collected at times 0 hour (before taking fluconazole), 1, 2, 4, 8, 12, and 24 hours (after taking fluconazole). Period II, on the day 15 of fluconazole 400 mg/day, blood samples were collected at 0, and 4 hours after fluconazole administration. Period III, when the patients had negative CSF culture, the patients would received fluconazole 200 mg/day, on the day 8 after fluconazole 200 mg/day therapy, blood samples were collected at 0, and 4 hours after the drug had been taken.

The blood samples were allowed to clot at room temperature, centrifuged at 2400 rpm for 6 minutes then serum samples were separated and kept at -20□C until analyzed.

Cerebrospinal Fluid (CSF) collection

CSF samples were collected and monitored for the conversion time in order to compare the efficacy of fluconazole treatment on cryptococcal meningitis between the two groups. After fluconazole 400 mg/day therapy had been started for 1, 2, 4, 6, and 8 weeks, CSF sample were collected until the cultures of *Cryptococcus neoformans* became negative. The CSF samples were sent to Chemical laboratory of Bamrasnaradura hospital to found the CSF component (protein, glucose, WBC, and number of fungal cell that positive india ink stain) and were sent to Microbiology laboratory of Bamrasnaradura hospital for *C. neoformans* isolation.

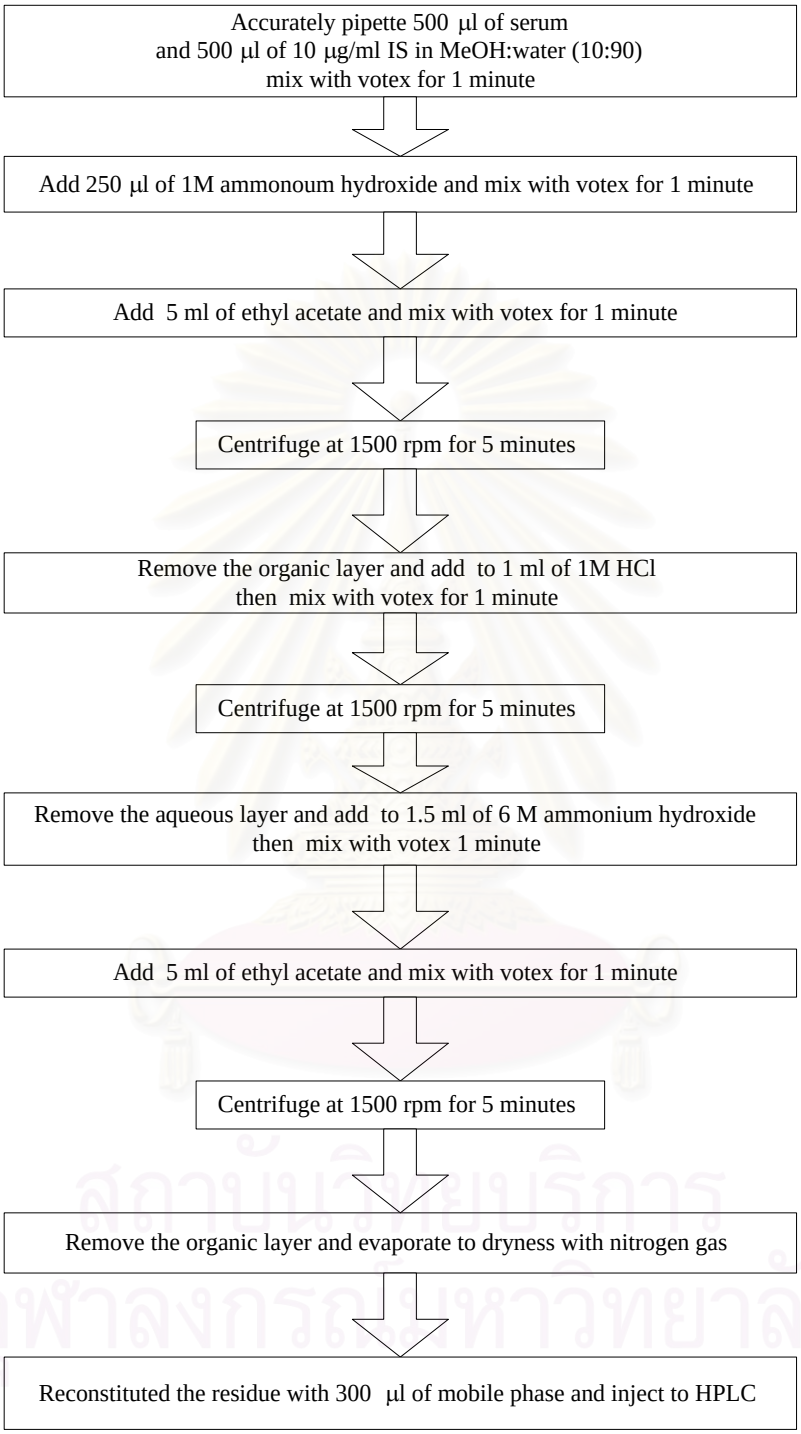
Figure 6: Flow chart of the study



Drug Assay

Concentrations of fluconazole in serum samples were quantified using the high performance liquid chromatography (HPLC). Phenacetin (1 mg/ml) was used as the internal standard (IS) and the serum samples extraction was modified from the method of Foulds et al[38]. The mobile phase was methanol (MeOH): 10 mM pH 7 phosphate buffer (50:50 v/v), at a flow rate 1 ml/min. The column was adsorbosphere C 18 (Waters Associates). The column effluent was monitored by UV detection at 260 ηm. The limit of detection of fluconazole was 1 ηg/ml. The method of serum samples extraction was shown in figure 7.

Figure 7: The method of sample extraction as modified from the method of Foulds[38]



Pharmacokinetic parameters

During period I of the samples collection, the complete-concentration-time profile of one dosing interval of fluconazole 400 mg could be generalized. There were 7 concentrations at various times during 24 hours dosing interval (0, 1, 2, 4, 8, 12, and 24 hours). In this period, the pharmacokinetic parameters of fluconazole: the maximum plasma concentration (C_{max}), time to reach C_{max} (T_{max}), the area under the concentration time ($AUC_{0-24)}$, absorption rate constant (Ka), elimination rate constant (Ke) and the half-life ($T_{1/2}$) were derived using program RSTRIP version 2.0 which is the program for compartmental modeling and kinetic analysis for PC. Clearance (Cl)and volume of distribution (Vd) were derived from equation 1 and 2 respectively.

$$\mathrm{Cl\,(L/hr)} = \frac{\mathrm{Dose}}{\mathrm{AUC}} \qquad \text{Equation 1}$$

$$\mathrm{Vd\,(L)} = \mathrm{Cl} \div \mathrm{Ke} \qquad \text{Equation 2}$$

During period II and III blood samples were collected again at time 0 hour and 4 hour. Ke were calculated from the negative slope of the semi-log concentration time curves generated from fluconazole concentrations at these two times for all three periods.

Statistical analysis

In order to compare the pharmacokinetic parameters between the two groups, we made the assumption that if the AUC obtain from the group of patients who receive fluconazole alone is at least 25% higher[13] than those obtain from the group of patients who receive rifampicin along with fluconazole, the interaction will be concluded as causing significant difference in pharmacokinetic parameters. Using two-sided test, an alpha level of 0.05, a power of 80 percent to detect a difference of 25 percent in AUC between the two groups, the analysis would required at least 11 patients per group.

To compare the conversion time of CSF cultures between the two groups, we assumed that the difference in conversion times of 2 weeks or longer[6] will be concluded as significant difference clinically. Using two-sided test, an alpha level of 0.05, a power of 80 percent to detect a true difference conversion times between the two groups, the analysis would required at least 20 patients per group.

General characteristics and information obtained from the subjects including laboratory data were recorded in case report forms. The data were analyzed using descriptive statistics, comparisons were performed by chi-square test or Fisher's exact test for categorical variables and student's t-test or ANOVA for continuous variables.

CHAPTER IV

RESULTS AND DISCUSSION

1. Study population

Between January 2001 and July 2001, 40 patients with AIDS associated cryptococcal meningitis were enrolled in this study during their admission in the medical ward at Bamrasnaradura hospital. All patients signed their consent to participate in this study. All forty patients received amphotericin B 0.7 mg/kg/day to treat cryptococcal, in which twenty of them also received rifampicin to treat tuberculosis. After received amphotericin B for 14 days, all patients were continued their treatment by receiving fluconazole 400 mg/day. Blood samples were collected periodically from the first twelve patients of the fluconazole alone group and the fluconazole plus rifampicin group, the fluconazole concentrations were analyzed and the pharmacokinetic parameters were then determined.

Demographic data

Of the 40 patients with AIDS-associated cryptococcal meningitis, 28 patients (70%) were male with a range of age 21-48 years and the mean age equal to 31.68 ± 6.31 years (mean ± S.D.). The top three occupations of patients were employee (45%), commercial (25%), and unemployed and government officer, which were in equal percentage (12.5%). Twenty-one patients (52.5%) were married while 17 patients (42.5%) and 1 patient (5%), which were single and widowed respectively. Mean hematocrit level was 32.04±5.94 percentage, the low hematocrit levels were common found in patients with AIDS. White blood cell count showed the mean which was equal to 4.6±1.998 ($x10^3$ cell/mm^3), which was within the normal range value. Both blood urea nitrogen (BUN) and creatinine were within normal range with the mean values equal to 13.65±6.21 mg/dl and 0.51-2.33 mg/dl, respectively. Demographic data were shown in Table 2.

Table 2: Demographic data of patients with AIDS-associated cryptococcal meningitis

Data	FLU (n=20)	FLU+RIF (n=20)	TOTAL (n=40)
Sex			
(no.(%) of patients)			
1. Male	14 (70)	14 (70)	28 (70)
2. Female	6 (30)	6 (30)	12 (30)
Age (years)			
Mean ± S.D.	32.25±5.4	31.40±6.85	31.68±6.31
Range	24-46	21-48	21-48
Occupation			
(no.(%) of patients)			
1. Unemployed	2(10)	3(15)	5(12.5)
2. Employee	11(55)	7(35)	18(45)
3. Commercial	5(25)	5(25)	10(25)
4. Agriculturist	0(0)	1(5)	1(2.5)
5. Government officer	2(10)	3(15)	5(12.5)
6. Student	0(0)	1(5)	1(2.5)
Marital status			
(no.(%) of patients)			
1. Single	8(40)	9(45)	17(42.5)
2. Married	11(55)	10(50)	21(52.5)
3. Widowed	1(5)	1(5)	2(5)
Base line laboratory			
1. Hematocrit (%)			
Mean ± S.D.	32.19±6.7	31.9±5.24	32.04±5.94
Range	17-42	24-40	17-42
2. White blood cell ($x10^3$ cell/mm^3)			
Mean ± S.D.	4.101±1.354	5.189±2.372	4.60±1.998
Range	1.7-7.1	2.6-12.6	1.7-12.6
3. BUN (mg/dl)			
Mean ± S.D.	13.8±5.00	13.5±7.36	13.6±6.21
Range	6-20	1-37	1-37
4. Creatinine(mg/dl)			
Mean ± S.D.	0.92±0.23	1.088±0.394	1.0045±0.3284
Range	0.51-1.48	0.6-2.33	0.51-2.33

2. The standard curve of Fluconazole in plasma

Standard fluconazole at various concentrations 1, 3, 5, 10, 15, 20, and 40 mg/L were added to blank plasma from normal subjects in order to make the standard curve. Phenacetin (1 mg/mL) was used as the internal standard (IS). After several steps of extraction according to the modified method of Foulds et. al., the samples were injected into HPLC. The retention time of fluconazole was about 5.3 minutes while that of the internal standard was about 8.9 minutes. The chromatograms were shown in figure 8, 9, 10 and 11.

Figure 8: Chromatogram of fluconazole and internal standard from standard solutions

Figure 9: Chromatogram of fluconazole and internal standard from blank plasma of normal subjects

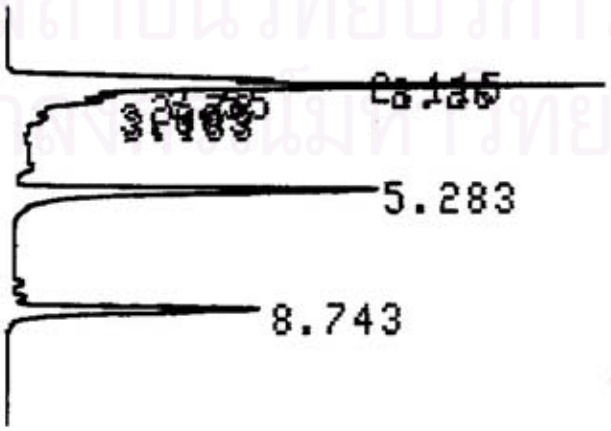


Figure 10: Chromatogram of fluconazole and internal standard from patient serum containing low fluconazole concentration (2.962 mg/L)

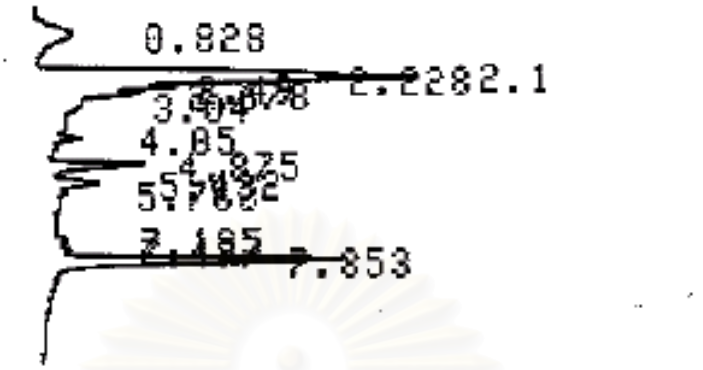


Figure 11: Chromatogram of fluconazole and internal standard from patients serum containing high fluconazole concentration (26.347 mg/L)

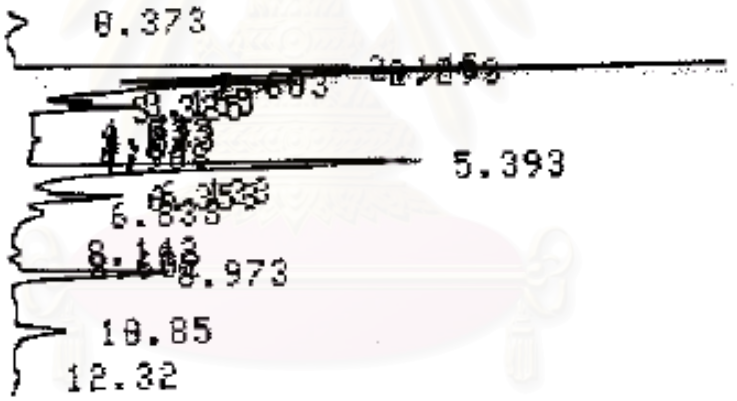
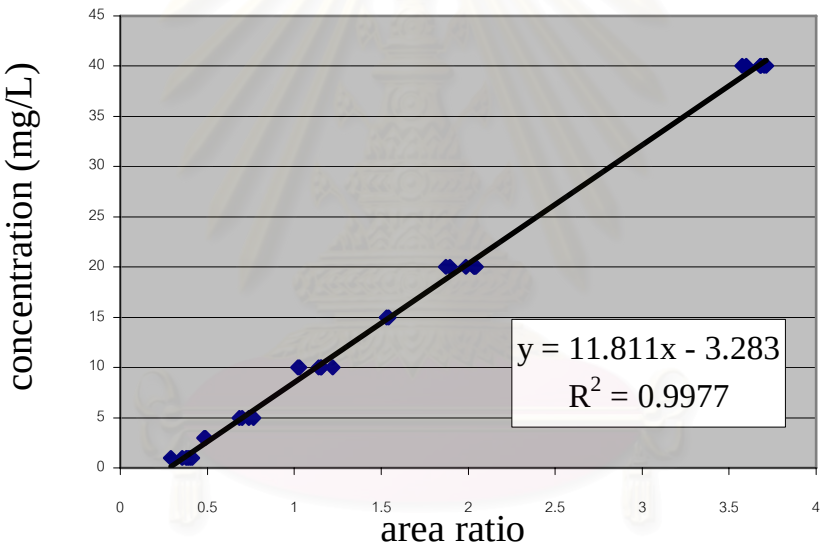


Figure 10 and 11 showed chromatograms of fluconazole and internal standard from patient serums containing low and high fluconazole concentrations respectively indicated that the method of extraction and analysis modified from the method of Foulds et. al could sucessfully separate fluconazole from the internal standard for either low or high concentrations.

The standard curve was created from the plot between fluconazole concentrations versus area ratios of fluconazole and internal standard from the chromatograms as show in figure 12. The equation of standard curve was $y = 11.811x – 3.283$ ($r^2 = 0.9977$). The coefficients of variation (%CV) between-run were used to make the precision of this standard curve. The %CV at concentrations of 1, 10, 20 and 40 mg/L were 12.09973, 6.926295, 3.887931, and 1.538223 respectively.

Figure 12: The standard curve of area ratio versus different concentrations of fluconazole



3. Pharmacokinetic interaction

3.1 Extent of interaction

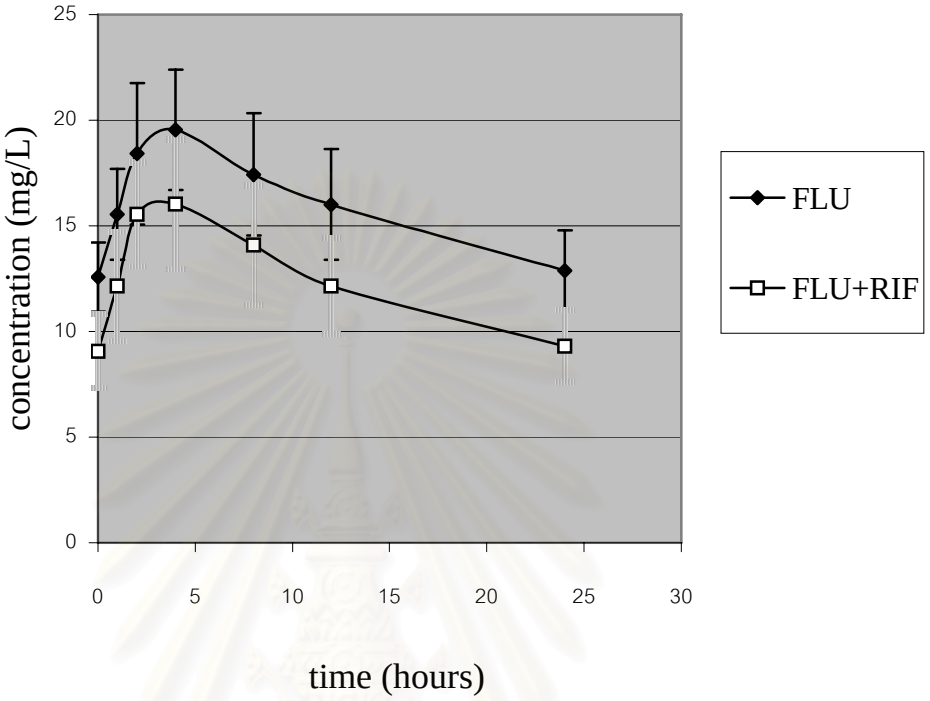
The blood samples were collected in three different periods from twelve patients of each groups (fluconazole alone and fluconazole plus rifampicin groups) at various times after the patients had received fluconazole. The first twelve patients out of the twenty patients who met the inclusion criteria of each group were selected. All patients in the concomitant drugs group had received rifampicin in oral dose of 600 mg/day at night for more than 2 weeks before fluconazole was administered.

Period I, on day eight after the administration of fluconazole 400 mg/day therapy, blood samples were collected at 0, 1, 2, 4, 8, 12, and 24 hours after fluconazole was administered. The means concentrations (Mean±S.D.) of fluconazole at various times of both groups were shown in the table 3 and figure 13. The mean concentrations (Mean±S.D.) of fluconazole for patients who received fluconazole alone at time 0, 1, 2, 4, 8, 12, 24 hours were 12.58±1.63, 15.54±2.14, 18.41±3.36, 19.56±2.85, 17.44±2.89, 16.01±2.62, and 12.88±1.90 mg/L respectively, while the concentrations were 9.07±1.74, 12.14±2.59, 15.56±2.45, 16.02±3.07, 14.09±2.80, 12.16±2.27, and 9.31±1.69 mg/L respectively for patients who received both fluconazole and rifampicin. The maximum of mean concentrations for both groups were appeared at 4 hours however, from individual data, there were 4 patients in fluconazole alone group, and 6 patients in the concomitant group whose maximum concentration were recorded in 2 hours (detail data was appendix D). The times to peak fluconazole concentration were previously reported to be within 0.5-6 hours after orally dose [9,11]. The concentrations between the two groups were significantly different at all times ($P < 0.05$). The range of fluconazole concentrations was 9.44-27.93 mg/L for fluconazole alone group and was 6.45-23.48 mg/L for the concomitant group. These mean concentrations were closed to those of the other studies which reported the range concentrations after fluconazole 400 mg/day to be about 10-20 mg/L [9, 11, 29, 31]

Table 3: Comparison of Fluconazole concentrations between Fluconazole Alone and Fluconazole with Rifampicin groups during period 1 (On day 8 after starting Fluconazole 400 mg/day therapy).

Time(hours)	Fluconazole Concentration (mg/L) Mean ± S.D. (Range)		*P*-value
	FLU (N=12)	FLU+RIF (N=12)	
0	12.58±1.63 (10.96-16.80)	9.07±1.74 (6.45-12.10)	0.000
1	15.54±2.14 (12.44-20.62)	12.14±2.59 (8.69-16.65)	0.002
2	18.41±3.36 (15.64-27.93)	15.56±2.45 (11.31-19.28)	0.026
4	19.56±2.85 (14.42-26.35)	16.02±3.07 (12.65-23.48)	0.008
8	17.44±2.89 (12.47-24.67)	14.09±2.80 (10.94-20.04)	0.009
12	16.01±2.62 (12.07-22.57)	12.16±2.27 (9.46-16.74)	0.001
24	12.88±1.90 (9.94-17.73)	9.31±1.69 (6.68-13.01)	0.000

Figure 13: Compared Fluconazole concentration versus time curves between fluconazole alone and fluconazole with rifampicin groups during period I (On day 8 after starting Fluconazole 400 mg/day therapy).



During period I, seven concentrations at various times during the same dosing interval (0, 1, 2, 4, 8, 12, and 24 hours) were collected and analyzed to obtain the complete pharmacokinetic profile after 400 mg/day of fluconazole. Pharmacokinetic parameter of fluconazole i.e., the maximum plasma concentration (C_{max}), time to reach C_{max} (T_{max}), the area under the concentration time ($AUC_{0-24)}$, absorption rate constant (Ka), elimination rate constant (Ke) and the half-life ($T_{1/2}$) were derived with the use of RSTRIP. It is a program for compartmental modeling and kinetic analysis for PC. The resulted from RSTRIP showed that the kinetics profiles of all patients in both groups could be explained by the 2 exponential terms. Clearance and Vd were calculated from equation 1 and 2. The results and compared of pharmacokinetic parameters of both groups were shown in table 4.

Table 4: Pharmacokinetic parameters between two groups

Pharmacokinetic parameters	Mean ± S.D.		Statistic	
	FLU (N=12)	FLU+RIF (N=12)	*P*-value	95% CI of the difference
1. Ke (hr^{-1})	0.0218±0.0024	0.0303±0.0036	0.000	(-0.011)-(-0.0059)
2. $T_{1/2}$ (hr)	32.27±4.64	23.17±2.78	0.000	5.86-12.34
3. AUC_{0-24} (mg.hr/L)	386.11±58.50	299.29±53.87	0.001	39.21-134.43
4. Tmax (hr)	3.945±1.16	3.67±.0.93	0.532	(-0.616)-1.159
5. Cmax (mg/L)	18.99±2.88	15.67±2.76	0.009	0.919-5.693
6. Ka (hr^{-1})	0.8286±0.5589	0.7856±0.2983	0.816	(-0.336)-0.4422
7.Clearance (L/hr)	1.0556±0.1452	1.3722±0.2205	0.000	(-0.475)-(-0.158)
8.Vd (L)	49.26±10.32	45.86±8.74	0.394	(-4.70)-11.49

As presented in table 4, this study found that the mean (Mean ± S.D.) Ke in the group of patients who received only fluconazole was 0.0218±0.0024 hr^{-1} while in the group of patients who received fluconazole concomitantly with rifampicin was 0.0303±0.0036 hr^{-1}. The mean (Mean ± S.D.) $T_{1/2}$ in the group of patients who received only fluconazole was 32.27±4.64 hrs while in the group who took fluconazole concomitantly with rifampicin was 23.17±2.78 hrs. From a statistical point of view, there were significant differences in Ke and $T_{1/2}$ ($P < 0.05$) between the two groups. The mean (Mean ± S.D.) area under the concentration-time curves (AUC_{0-24}) in the group of patient who did not receive and who received rifampicin concomitantly were 386.11±58.50 mg.hr/L and 299.29±53.87 mg.hr/L respectively

which was significantly different between the two group (P = 0.001). All patients in both groups displayed the time to reach maximum concentration (Tmax) to be within 4 hours after dosing of fluconazole. There were no significant deference of Tmax between the two groups. Tmax of all patients in this study were similar to those mentioned in other studies which revealed fluconazole capsule to be rapidly absorbed with a time between 0.5-6 hours.[9,11] Comparison between maximum concentrations (Cmax) found in this study showed that the mean (Mean ± S.D.) was 18.99±2.88 mg/L in the patients group who did not receive rifampicin and was 15.67±2.76 mg/L in the group who received fluconazole concomitantly with rifampicin. These values were significantly different between the two groups (P = 0.009). The comparison in absorption rate constants (Ka) found that the mean (Mean ± S.D.) Ka in the group of patients who did not receive rifampicin was 0.8286±0.5589 hr^{-1} and was 0.7856±0.2983 hr^{-1} in the group who received rifampicin concomitantly. There were no significant difference in Ka between the two groups (P = 0.816) indicated that the bioavailability between two groups were similar. The mean clearance (Mean ± S.D.) was 1.0556±0.1452 L/hr in the patients group who did not receive rifampicin and was 1.3722±0.2205 L/hr in the group who received fluconazole concomitantly with rifampicin. These values were significantly different between the two groups (P = 0.000). The comparison in volume of distribution (Vd) found that the mean (Mean ± S.D.) Ka in the group of patients who did not receive rifampicin was 49.26±10.32 L and was 45.86±8.74 L in the group who received rifampicin concomitantly. There were no significant difference in Vd between the two groups (P = 0.394)

The pharmacokinetic parameters of fluconazole presented in this study were derived from the concentrations of fluconazole, which were at steady state. This was different from other studies, which were accomplished on a single dose of fluconazole. This study found that concomitant administration of rifampicin with fluconazole resulted in significantly change in the pharmacokinetic parameters of fluconazole. These changes included 39.08 % increase in Ke, 28.19 % decrease in half-life, 22.48 % decrease in AUC_{0-24}, 17.41 % decrease in the maximum concentration (Cmax) and 29.99 % increase in clearance of fluconazole. In the open-label randomized placebo-control study of Apseloff et. al.[13] in 16 healthy volunteers, a single dose of fluconazole 200 mg/day concomitant by administered with rifampicin

600 mg/day resulted in significant increased in the elimination rate of drug which were characterized by a 23% decrease in $AUC_{(0-\infty)}$ (P = 0.0018), 25% increase in Ke and the decrease of elimination half life from 32 to 26 hours (19% decrease). In the study of Lazar et. al.[14], concomitant administration of fluconazole with rifampicin resulted in a 22% decrease in the mean half-life and 23% decrease in the mean $AUC_{(0-\infty)}$ (P<0.002) from baseline values in healthy volunteers who were administered rifampicin with fluconazole 200 mg concurrently. Another study[35] about the influence of rifampicin 1200 mg/day co-administration on the pharmacokinetics of fluconazole 100-200 mg/day in two critically ill patients as compared with those obtained after fluconazole without rifampicin on three critically ill patients. The study reported 52% decrease in AUC and 93% increase in total clearance of fluconazole, accepting limitations of the study design, the authors suggested an interaction of greater significance than reported in volunteers, with the potential for poor anti-fugal treatment outcomes.

Although fluconazole is excreted mainly unchanged in urine, in human the unchanged fraction of the drug in urine is in the range of 64 to 90%, 11.4% of an administered dose was recovered in urine as metabolites and very little metabolism occurred during the first pass through the liver after gastrointestinal absorption. When concomitant administer fluconazole with rifampicin, which is a potent inducer of cytochrome P450 enzyme. Rifampicin enhances the elimination of fluconazole, most likely by inducing cytochrome P-450 in the liver. This study found change in the elimination of fluconazole caused by rifampicin since all pharmacokinetic parameters (Cmax, AUC, Ke, half-life, clearance) except Tmax, Ka and Vd changed significantly when rifampicin was concomitantly administered with fluconazole. In clinical consideration, due to approximately 22.48 % decrease in AUC, 17.41 % decrease in Cmax, 28.19 % decrease in half-life and 29.99 % increase in clearance of fluconazole detected in this study, increasing in the dosages of fluconazole may be required in the patients who also received rifampicin for the treatment of tuberculosis along with fluconazole for cryptococcal meningitis.

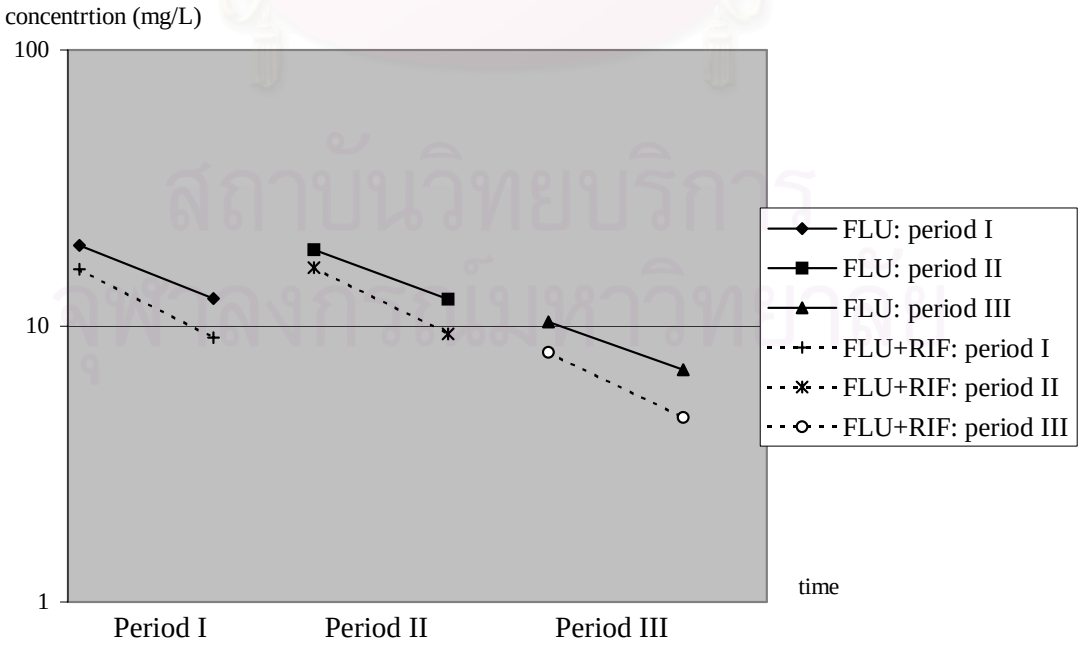
3.2 The effect of time and doses on interaction

Period II, on day 15 of fluconazole 400 mg/day therapy, two blood samples were collected at the time 0, and 4 hours after fluconazole administration from the same twelve patients of each group. The mean fluconazole concentration (Mean±S.D.) of patients who received fluconazole alone at times 0 hour and 4 hour were 12.51±1.93 and 18.89±2.91 mg/L respectively, while for the group who received fluconazole concomitant with rifampicin the concentration at times 0 hours and 4 hours were 9.36±1.49 and 16.25±2.75 mg/L respectively. In period III, after the conversion of CSF, fluconazole dosage of all patients was changed to 200 mg/day. Blood samples were again collected from the same twelve patients of each group at time 2 time 0, and 4 hours on the day 8 after starting the dosing of fluconazole 200 mg/day. The mean concentrations (Mean±S.D.) of fluconazole at time 0 and 4 of patients who received only fluconazole were 6.95±1.52 and 10.34±2.12 mg/L respectively, while in the group who received both fluconazole and rifampicin were 4.66±0.83 and 8.03±1.30 mg/L respectively. The range of fluconazole alone group was 5.00-14.78 mg/L and the range for concomitant group was 2.96-10.93 mg/L. These ranges of fluconazole concentrations after received fluconazole 200 mg/day obtained from this study were similar to those reported in other studies. In other studies[9,11] the range of fluconazole concentrations at steady state after administration of fluconazole 200 mg/day alone was 5-10 mg/L. Another study[13] reported the maximum concentration, after a single dose of fluconazole 200 mg/day was administered in a group of healthy volunteers either received fluconazole alone or received fluconazole concomitant with rifampicin, to be 3.21±0.38 and 3.14±0.41 mg/L respectively. The concentrations in aforementioned study was lower than those found in this study since in this study the concentrations was at steady state, therefore the peak plasma concentration should be increase approximately 2.5 times[9,11] after a single dose. The comparison of fluconazole concentrations between two groups during period I (On day 8 of fluconazole 400 mg), period II (On day 15 of fluconazole 400 mg) and period III (On day 8 of fluconazole 200 mg) were shown in table 5 and figure 14.

Table 5: Comparison of fluconazole concentrations between fluconazole alone and fluconazole with rifampicin groups at three different periods

Group	Flluconazole concentration Mean ± S.D. (mg/L) (Range)		
	Period I	**Period II**	**Period III**
FLU			
Time 0	12.58±1.63	12.51±1.93	6.95±1.52
	(10.96-16.80)	(10.10-17.58)	(5.00-10.12)
Time 4	19.56±2.85	18.89±2.91	10.34±2.12
	(14.42-26.35)	(14.43-26.25)	(7.37-14.78)
FLU+RIF			
Time 0	9.07±1.74	9.36±1.49	4.66±0.83
	(6.45-12.10)	(6.45-12.10)	(2.96-6.26)
Time 4	16.02±3.07	16.25±2.75	8.03±1.30
	(12.65-23.48)	(12.88-23.40)	(5.60-10.93)

Figure 14: Fluconazole concentration versus time curves (log scale) of fluconazole alone and fluconazole with rifampicin groups at three different periods



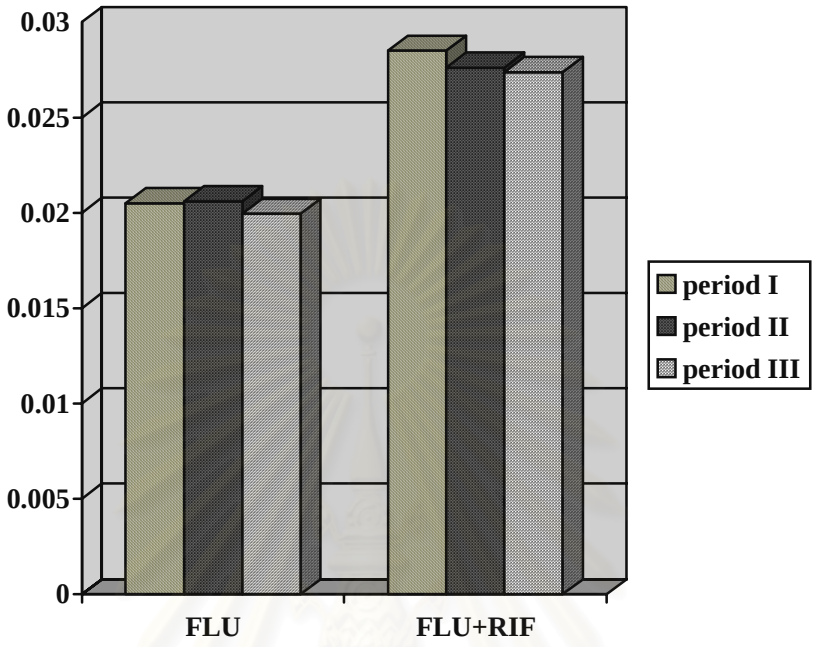
As shown in table 5 and figure 14, fluconazole concentrations in period III were about 0.5 times lower than concentration in period I and II for both groups since the dosage of fluconazole was decreased by one half. There were no significant different in concentrations at either time 0 hour or 4 hour between the first two periods within the same group since the concentrations of fluconazole should reach the steady state within 7 days[9, 11, 29, 31] of regularly administration of the same dosage regimen.

During period I, period II and period III, fluconazole concentrations of the two times intervals (0 hour and 4 hour) were used to calculate Ke from the negative slope curve (using semi-log concentration and time curve). The mean (Mean±S.D.) Ke from period I (On day 8 of Fluconazole 400 mg/day), period II (On day 15 of Fluconazole 400 mg/day) and period III (On day 8 of Fluconazole 200 mg/day) were compared as shown in table 6 and figure 15. There was no significant difference between the Ke at the three different periods in both groups (P>0.05 by Repeated-Measures Analysis of Variance). Different times and the different doses of fluconazole did not affect the value of Ke obtained from the steady state concentration of fluconazole in both groups.

Table 6: Comparison of elimination rate constants (Ke) obtained from the blood samples collected at three different period

Group	**Ke (hr^{-1}) (Mean±S.D.)**			**Statistic (within group)**
	Period 1 On day 8 of Fluconazole 400 mg/day	**Period 2** On day 15 of Fluconazole 400 mg/day	**Period 3** On day 8 of Fluconazole 200 mg/day	
FLU	0.0205±0.00173	0.0206±0.00197	0.01995±0.00116	P>0.05
FLU+RIF	0.0285±0.00700	0.0276±0.00341	0.02737±0.00233	P>0.05
Statistic (between group)	P=0.001	P=0.000	P=0.000	

Figure 15: Comparison of elimination rate constants (Ke) obtained from the blood samples collected at three different period



However, as presented in table 6 and figure 15, the Ke of fluconazole in the patients who received rifampicin concomitantly with fluconazole were 39.02%, 33.98% and 37.19% higher during period I, period II and period III respectively than those of the patients who received fluconazole alone. The extent of interaction of rifampicin on fluconazole did not change with times and doses of fluconazole indicated that the extent of interaction was complete and stable.

4. The effect of interaction on clinical efficacy

4.1 Cerebrospinal Fluid (CSF) component

Comparisons of CSF components between the two groups during therapy were shown in table 7. CSF components of most patients changed to the normal range within 6 weeks of therapy. There were no significant differences of CSF components between the two groups. The abnormal mean protein decreased to normal ranges (≤ 45 mg/dl) and the glucose level increased to normal ranges (≥ 40 mg/dl) within 3 weeks of therapy. CSF white blood cell and OP trend slowly decreased to normal range in both groups.

Table 7: Comparisons of CSF components between the two groups

Lumbar puncture	group	baseline	Week 3	Week4	Week6
1.OP (mm H_2O)	FLU	414.35±113.78 (N = 20)	275.10±94.56 (N = 20)	212.92±50.69 (N = 14)	185.00±7.07 (N = 2)
	FLU+RIF	404.20±81.05 (N = 20)	255.50±69.26 (N = 20)	206.31±23.98 (N = 12)	198.50±16.26 (N = 2)
2.Protein (mg/dl)	FLU	50.9±22.02 (N = 20)	41.14±9.93 (N = 20)	37.21±8.22 (N = 14)	35.25±14.50 (N = 2)
	FLU+RIF	49.94±16.54 (N = 20)	46.41±19.99 (N = 20)	41.40±5.63 (N = 12)	40.50±0.00 (N = 2)
3.Glucose (mg/dl)	FLU	40.65±8.49 (N = 20)	47.42±6.44 (N = 20)	51.77±8.41 (N = 14)	42.75±3.18 (N = 2)
	FLU+RIF	40.78±11.59 (N = 20)	47.51±6.25 (N = 20)	48.27±6.13 (N = 12)	44.50±8.49 (N = 2)
4.WBC (cells/mm^3)	FLU	23.50±23.52 (N = 20)	15.20±15.62 (N = 20)	4.77±4.04 (N = 14)	0.00 (N = 2)
	FLU+RIF	22.70±21.51 (N = 20)	8.55±7.22 (N = 20)	3.23±3.54 (N = 12)	0.50±0.71 (N = 2)
5.No. of fungus cell (cells/HPF)	FLU	13.95±14.48 (N = 20)	3.50±2.93 (N = 20)	1.31±1.03 (N = 14)	0.00 (N = 2)
	FLU+RIF	16.75±12.89 (N = 20)	7.60±7.47 (N = 20)	2.38±2.60 (N = 12)	0.00 (N = 2)

4.2 The rate at which CSF converse to negative cultures

The percentages of patients with each CSF conversion rates were shown in table 8. All patients had negative CSF culture within 6 weeks. There were no significant difference in conversion rates of CSF culture between two groups (P = 0.792 by Pearson chi-square). The median times that negative CSF culture was at 4 weeks in both groups. This median times of conversion rate was similar in others studies in the patients who received the same regimen to treatment cryptococcal meningitis; amphotericin B in initial therapy 2 weeks and follow with fluconazole 400 mg/day in a consideration therapy.[4-8]

Table 8: The rate at which the CSF converse to negative cultures of patients with AIDS associated cryptococcal meningitis

Rate of conversion (weeks)	No. (%) of patients		
	FLU (N = 20)	FLU + RIF (N = 20)	TOTAL (N = 40)
3	6 (30)	8 (40)	14 (35)
4	12 (60)	10 (50)	22 (55)
6	2 (10)	2 (10)	4 (10)

4.3 Relationship between the pharmacokinetic parameters of fluconazole and the time to negative CSF culture

In this study, all patients in both groups received the same therapy, i.e., they received amphotericin B in intravenous dosage of 0.7 mg/kg/day for 2 weeks followed by fluconazole 400 mg/day until the CSF cultures became negative. The conversion rate of CSF culture was within 6 weeks. The median time that CSF culture became negative with this study therapy was 4 weeks. Comparison of fluconazole pharmacokinetic parameters between the patients who had the CSF conversion rate at various times (at 3 weeks, 4 weeks and 6 weeks) were shown in table 9. Neither pharmacokinetic parameters showed any significant different in three groups of patients which might due to the anti-fungal effect caused by amphotericin B in the initial step of therapy during the first 2 weeks. Therefore, the pharmacokinetic parameters of fluconazole were not related to the conversion time to negative CSF culture.

Table 9: Comparison of the pharmacokinetic parameters of fluconazole between groups of patients who had the CSF conversion rates at various times (3 weeks, 4 weeks and 6 weeks)

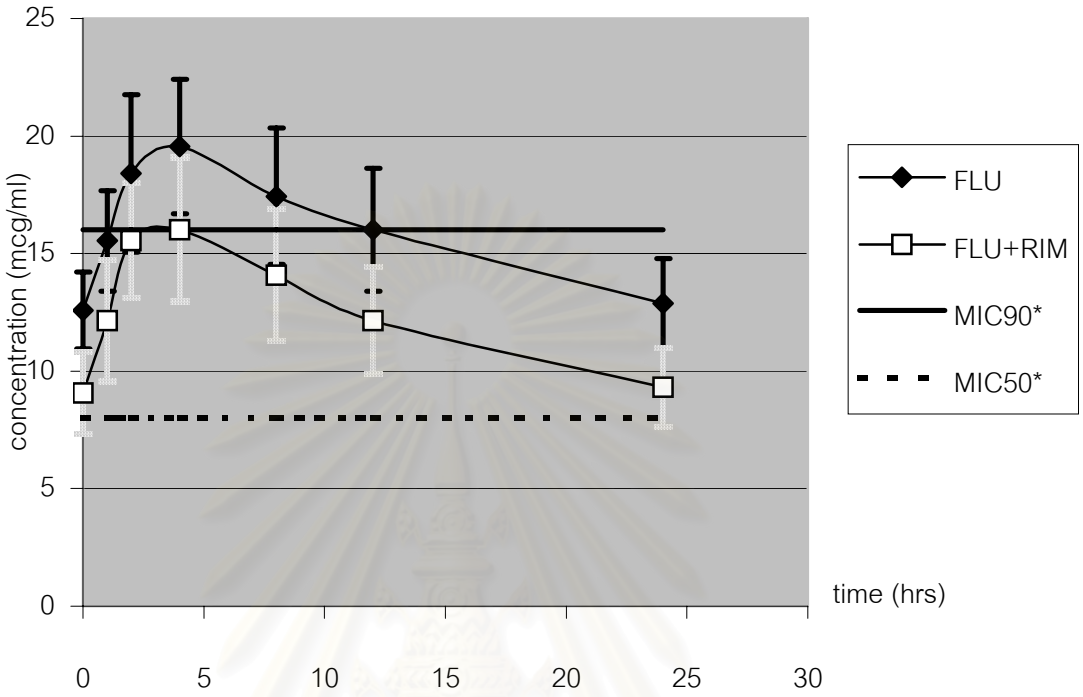
Pharmacokinetic parameters	**Conversion time Mean ± S.D.**			**Statistic** (*P*-value)
	3 weeks (N = 9)	**4 weeks** (N=13)	**6 weeks** (N=2)	
1. Cmax (mg/L)	17.60±3.12	17.33±3.59	16.24±2.21	0.875
2. Tmax (hours)	4.04±0.99	3.60±1.14	4.13±0.26	0.573
3. AUC (mg.hr/L)	342.90±69.30	344.48±77.07	330.18±68.81	0.968
4. half life (hours)	24.75±5.19	28.84±3.22	33.82±17.58	0.087
5. Ke (hr^{-1})	0.0291±0.0057	0.0243±0.0029	0.0237±0.0123	0.092
6. Ka (hr^{-1})	0.7046±0.33	0.9017±0.52	0.6537±0.16	0.532
7. Clearance (L/hr)	1.1458±0.185	1.1213±0.260	1.5281±0.205	0.133
8. Vd (L)	46.10±6.60	47.66±11.69	53.46±1.34	0.632

4.4 The fluconazole concentration versus MIC

In other study[43] of fluconazole susceptibility *in vitro* activity the MIC (90) range against 566 clinical isolates of *C. neoformans* was 8-16 mg/L. Another study[42] reported the range of MIC of 23 strains of *C. neoformans* isolated from CSF of 16 AIDS patients with meningitis or encephalitis, to be 2-8 mg/L. One more study from a total of 28 isolates of C. neoformans from 25 AIDS patients [41] reported fluconazole MIC at which 50% and 90% of the isolates were inhibited [MIC (50) and MIC (90)], to be 4 mg/L and 16 mg/L respectively. And the personal study in Bamrasnaradura hospital Thailand reported the range of MIC of 29 strains of *C. neoformans* isolated from CSF of AIDS patients were 4-16 mg/L, MIC (50) was 8 mg/L, MIC (90) was 16 mg/L and the mean MIC was 8.55 mg/L.

Period I, on day eight after the administration of fluconazole 400 mg/day therapy, blood samples were collected at 0, 1, 2, 4, 8, 12, and 24 hours after fluconazole was administered. The means concentrations (Mean±S.D.) of fluconazole at various times of both groups were shown in the table 3. The mean concentrations (Mean±S.D.) of fluconazole for patients who received fluconazole alone at time 0, 1, 2, 4, 8, 12, 24 hours were 12.58±1.63, 15.54±2.14, 18.41±3.36, 19.56±2.85, 17.44±2.89, 16.01±2.62, and 12.88±1.90 mg/L respectively, while the concentrations were 9.07±1.74, 12.14±2.59, 15.56±2.45, 16.02±3.07, 14.09±2.80, 12.16±2.27, and 9.31±1.69 mg/L respectively for patients who received both fluconazole and rifampicin. Although the mean concentrations in the patients who received concomitantly rifampicin with fluconazole had lower concentrations at all times but all of the concentrations in both groups were above the minimum inhibitory concentrations (MIC50) range of *C. neoformans* as shown in figure 16.

Figure 16: Fluconazole concentrations in period I versus MIC

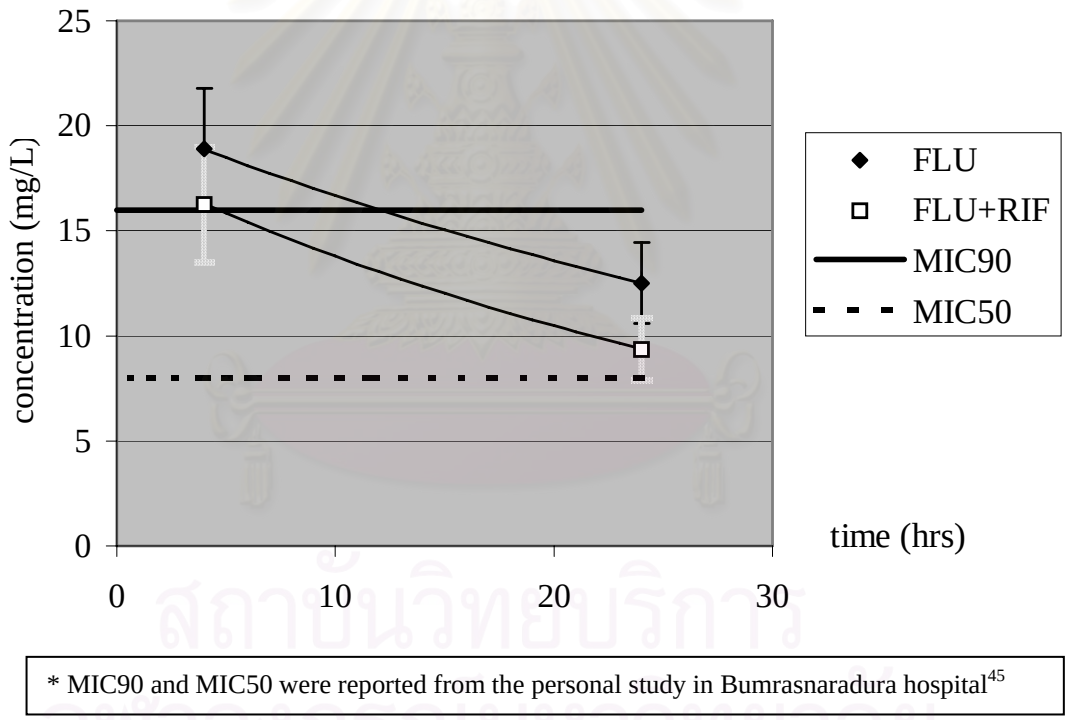


* MIC90 and MIC50 were reported from the personal study in Bumrasnaradura hospital[45]

Since the concentrations of fluconazole in both groups were above MIC, therefore the clinical outcomes (such as the CSF component, the rate at which CSF converse to negative culture) were not significantly different between the two groups.

Period II, on day 15 of fluconazole 400 mg/day therapy, two blood samples were collected at the time 0, and 4 hours after fluconazole administration from the same twelve patients of each group. The mean fluconazole concentration (Mean±S.D.) of patients who received fluconazole alone at times 0 hour and 4 hour were 12.51±1.93 and 18.89±2.91 mg/L respectively, while for the group who received fluconazole concomitant with rifampicin the concentration at times 0 hours and 4 hours were 9.36±1.49 and 16.25±2.75 mg/L respectively. Same as the period I, all concentrations obtained in period II of both groups were above the minimum inhibitory concentrations (MIC50) range of *C. neoformans* as shown in figure 17.

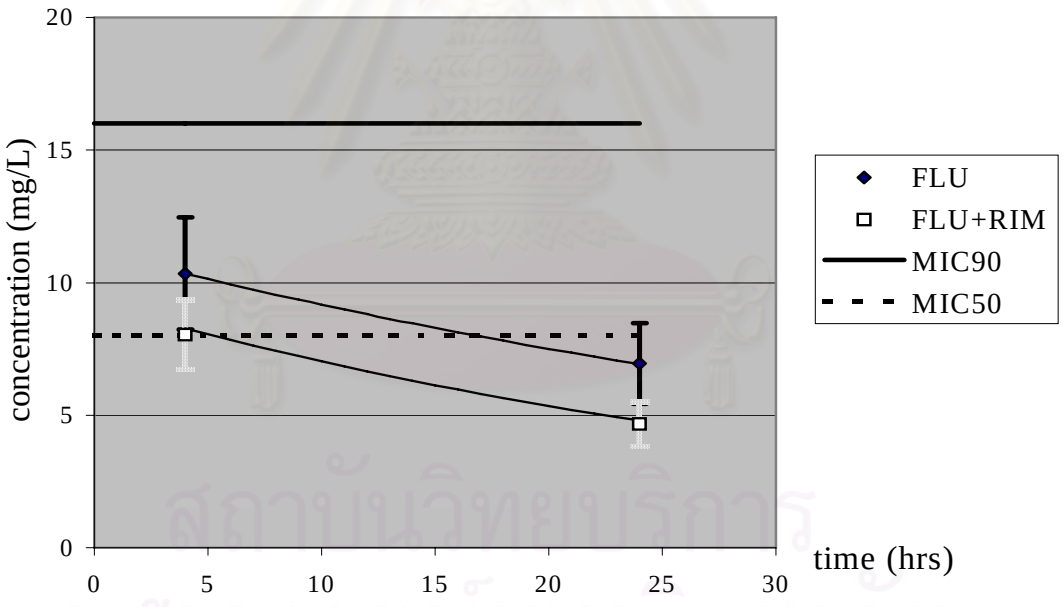
Figure 17: Fluconazole concentrations in period II versus MIC



* MIC90 and MIC50 were reported from the personal study in Bumrasnaradura hospital[45]

In period III, after the conversion of CSF, fluconazole dosage of all patients was changed to 200 mg/day. Blood samples were again collected from the same twelve patients of each group at time 2 time 0, and 4 hours on the day 8 after starting the dosing of fluconazole 200 mg/day. The mean concentrations (Mean±S.D.) of fluconazole at time 0 and 4 of patients who received only fluconazole were 6.95±1.52 and 10.34±2.12 mg/L respectively, while in the group who received both fluconazole and rifampicin were 4.66±0.83 and 8.03±1.30 mg/L respectively. The range of fluconazole alone group was 5.00-14.78 mg/L and the range for concomitant group was 2.96-10.93 mg/L. The concentrations in the group of patients who received fluconazole concomitant with rifampicin were lower than the MIC50 range of *C. neoformans* at all times as shown in figure 18.

Figure 18: Fluconazole concentrations in period III versus MIC



* MIC90 and MIC50 were reported from the personal study in Bumrasnaradura hospital[45]

During period III, fluconazole 200 mg/day was given to the patients in order to prophylaxis cryptococcal meningitis, therefore, the concentrations of fluconazole was expected to be able to prevent cryptococcal meningitis. Since the fluconazole concentrations were lower than MIC at all times in the group of patients who received fluconazole concomitantly with rifampicin, long term (6 months to one year) monitoring for the recurrent of cryptococcal meningitis may be required before any conclusion could be made on the effect of rifampicin interaction on the clinical outcomes of fluconazole. Rifampicin concomitantly administered with fluconazole caused significantly changed in the pharmacokinetics of fluconazole while no immediate clinical effects could be observed. However, whether or not these changes in pharmacokinetics will affect long term clinical outcomes of fluconazole, required farther studies.

CHAPTER V

CONCLUSION

1. Study population

Forty patients were enrolled in this study, twenty patients received fluconazole alone (FLU) while the other twenty patients concomitantly administered rifampicin along with fluconazole (FLU+RIF).

Demographic data

Of the 40 patients with AIDS-associated cryptococcal meningitis, 28 patients (70%) were male. The range of age was 21-48 years and the mean age was 31.68±6.31 years (Mean±S.D.). The three most common occupations of patients were employee (45%), commercial (25%) and unemployed and government officer in equal percent (12.5%). Baseline laboratory tests found showed the mean hematocrit level to be 32.04±5.94 percent, the mean white blood cell count to be 4.6±1.998 ($x10^3$ cell/mm^3) while the mean BUN and serum creatinine were 13.65±6.21 mg/dl and 0.51±2.33 mg/dl respectively.

2. The standard curve for plasma fluconazole concentrations

The HPLC method for analysis of fluconazole concentrations in plasma was developed by modified from those of Foulds et al. method[38]. The standard curve was developed by using various concentrations of fluconazole 1, 3, 5, 10, 15, 20 and 40 μg/ml and Phenacetin (1 mg/ml) was used as the internal standard. The retention time of fluconazole was 5.8 minutes while the internal standard showed up at 9.3 minutes. The correlation between concentrations and area ratio (of fluconazole and internal standard) was $y = 11.811x - 3.283$ ($r^2 = 0.9977$).

3. Pharmacokinetic interaction

3.1 Extent of interaction

This study found that concomitant administration of fluconazole and rifampicin resulted in significant change in the pharmacokinetic parameters of fluconazole. These changes included 39.08 % increase in Ke, 28.19 % decrease in half-life, 22.48 % decrease in AUC_{0-24}, 17.41 % decrease the maximum concentration (Cmax) and 29.99% increase clearance (*P*= 0.000, 0.000, 0.001, 0.009, 0.00 respectively).

3.2 The effect of time and doses on interaction

The mean Ke obtained from period I (On day 8 of Fluconazole 400 mg/day), period II (On day 15 of Fluconazole 400 mg/day) and period III (On day 8 of Fluconazole 200 mg/day) were not significantly different among the three periods in both groups (*P*>0.05 by Repeated-Measures Analysis of Variance). Different times and the different doses of fluconazole did not affect the value of Ke obtained from the steady state concentrations of fluconazole in both groups indicated that the interaction of rifampicin on fluconazole was complete and stable since period I.

4. The effect of interaction on clinical efficacy

4.1 Cerebrospinal Fluid (CSF) component

There were no significant differences in CSF components between the two groups. The abnormal mean protein and mean glucose became within normal range in week 3 of the therapy (week 1 after received fluconazole 400 mg/day). CSF white blood cell count and OP slowly decrease to normal range in both groups.

4.2 The rate at which CSF converse to negative cultures

CSF culture of all patients showed negative results within 6 weeks. The median times that negative CSF culture was at 4 weeks in both groups. There were no significant differences in conversion rates of CSF cultures between two groups (P = 0.792 by Pearson chi-square)

4.3 Relationship between the pharmacokinetic parameters of fluconazole and the time to negative CSF culture

Comparison of fluconazole pharmacokinetic parameters between groups of patients who had a conversion rate at various times (3 weeks, 4 weeks and 6 weeks) showed no significant differences in any pharmacokinetic parameters.

4.4 The fluconazole concentration versus MIC

Although the mean concnetration in the patients who received concomitantly rifampicin with fluconazole had lower concentration at every time in period I and II but all of the concentrations in both groups were above the minimum inhibitory concentrations (MIC) range of *C. neoformans*. In period III, the concentrations in the group of patients who received fluconazole concomitant with rifampicin were lower than the MIC indicating that dosage increment may be required or recurrent rates of cryptococcal meningitis could be high. However, long term studies should be performed.

In summary, co-administration of rifampicin with fluconazole caused significant changes in the pharmacokinetic parameters of fluconazole including Ke, half-life, AUC, Cmax and clearance. Because rifampicin is a potential hepatic microsomal enzymes inducer, and it has been shown to accelerate the metabolism of fluconazole.

In this study, the drug interaction did not cause any significant changes in the clinical outcome when fluconazole was used to treat cryptococcal meningitis, as shown by no significant differences in conversion rate of CSF cultures whether or not rifampicin were concomitantly administered with fluconazole. There were no relationships of any pharmacokinetic parameters with the time to negative CSF culture. Since amphotericin B was given during the first 2 weeks in the initial therapy of cryptococcal meningitis and fluconazole concentration may cause improvement in the clinical outcomes even though the concentrations and pharmacokinetic parameters of fluconazole changed significantly when co-administered with rifampicin. The dose 200 mg/day of fluconazole is used as a prophylaxis for cryptococcal meningitis, the clinical response to fungal infection should be monitored closely in long term for recurrent rates of cryptococcal meningitis since the concentrations of fluconazole which were lower than MIC when concomitantly administered with rifampicin could lead to poor outcomes. For serious infections, one might consider increase in the dosage of fluconazole for approximately 30%.

REFERENCE

1. Peiperl, L. Current clinical strategies manual of HIV/AID therapy. Singapore : Info accessand distribution PTE, 1996: 49-56.
2. กองระบาทวิทยา. สถานการณ์ผู้ป่วยเอดส์ของประเทศไทย ณ วันที่ 31 มกราคม พ.ศ.2543. รายงานการเฝ้าระวังโรคประจำเดือน2543 31 (2543) : 25-31.
3. กระทรวงสาธารณสุข. แผนป้องกันและแก้ไขปัญหาเอดส์แห่งชาติ พ.ศ. 2545-2549. สรรพ-สารวงการแพทย์ 89 (2544): 9
4. Powderly, W.G. Cryptococcal meningitis and AIDS. Clinical Infectious Diseases 17 (1993) : 837-842.
5. Dismukes, W.E. Management of cryptococcosis. Clinical Infectious Diseases 17 Suppl 2 : S507-S512.
6. Van der Horst, C.M., and et al. Treatment of cryptococcal meningitis associated with acquired immunodeficiency syndrome. N Eng J Med 377 (1997) : 15-21.
7. Powderly, W.G. Recent advances in the management of cryptococcal meningitis in patients with AIDS. Clinical Infectious Diseases 17 Suppl 2 : S507-S512.
8. Carver, P. L. Invasive fungal infections. in DiPiro, J.T., et al.,ed, Pharmacotherapy a pathophysiological approach, 1839-1866. Stamford, CT : Appleton&Lange, 1999.
9. Goa, K. L., and Barradell, L. B. Fluconazole: An update of its pharmacokinetic properties and therapeutic use in major superficial mycoses in immunocompeomised patients. Drugs 50 (1995) : 658-690.
10. Lomaestro, B. M., and Piatek, M. A. Update on drug interactions with azole antifungal agents. The Annals of Pharmacotherapy 32 (1998) : 915-928.
11. Susan, M., Grant and Stephen, P. Clissold. Fluconazole: A Review of its Pharmacodynamic and Pharmacokinetic Properties, and Therapeutic Potential in Superficial and Systemic Mycoses. Drugs 39 (1990) : 877-916.
12. Coker, R. J., and et al. Interaction between fluconazole and rifampicin. BMJ 301 (1990) : 818.

13. Apseloff, G., and et al. Induction of Fluconazole Metabolism by Rifampin: In Vivo Study in Humans. J Clin Pharmacol 31 (1991) : 358-361.

14. Lazar, J. D., and Wilner, K. D. Drug Interaction with Fluconazole. Reviews of Infectionus Diseases 12 (1990) : S327-S333.

15. Mulanovich, V.E., and Saag, M.S. Cryptococcal meningitis. In Berger, J. R., and Levy, R.M. (eds), AIDS and the nervous system. 661-675. 2nd edition. Philadelphia: Lippincott-Raven Publishers, 1997.

16. Drpught, E., and Dupont, B. Cryptococcosis. In Jabobs, P.H., and Nanall, L. (eds), Antifugal drug therapy: A complete guide for the practitioner, 143-164. New York: Marcel Dekker, 1990.

17. Graybill, J.R., Cryptococcus neoformans. In Gorbach, S., Bartlett, J.G., and Blacklow, N.R. (eds), Infectous Disease, 1895-1899. Philadelphia: W.B.saunder Company, 1992.

18. ศมนีย์ ศุขรุ่งเรือง. การวินิจฉัยทางห้องปฏิบัติการของการติดเชื้อราในอวัยวะตามระบบ. ใน นลินี อัศวโภคี (บรรณาธิการ), การรักษาด้วยวิธีการในปัจจุบันสำหรับโรคติดเชื้อที่พบบ่อย, หน้า 269-274. พิมพ์ครั้งที่ 4. กรุงเทพมหานคร : ที พี พริ้นท์, 2537.

19. เบญจวรรณ รุ่งปิตะรังสี และ วันงาม กองเกตุใหญ่. การตรวจน้ำไขสันหลัง. ภุมรา ตาละลักษณ์ และคนอื่นๆ (บรรณาธิการ), พยาธิวิทยาคลินิก, 139-150. กรุงเทพฯ : โรงพิมพ์เรือนแก้ว, 2538.

20. สุรพล สุวรรณกูล, บุญมี สถาปัตยวงศ์ และ สมสิทธิ์ ตันศุภสวัสดิกุล. ประสบการณ์ในการดูแลรักษาโรคติดเชื้อ HIV และเอดส์. ใน นลินี อัศวโภคิ และคนอื่นๆ (บรรณาธิการ), ประสบการณ์ด้านโรคติดเชื้อในประเทศไทย, 51-62. กรุงเทพฯ : โฮลิสติก, 2542.

21. สุทธิพันธ์ จิตพิมลมาศ และ วีรจิคค์โชติมงคล. การติดเชื้อคริปโตค็อกคัส ในระบบประสาทส่วนกลาง. ใน พงษ์ศักดิ์ วิสุทธิพันธ์, สุรางค์ เจียมจรรยา และ ดิษยา รัตนากร (บรรณาธิการ), โรคติดเชื้อของระบบประสาทในประเทศเขตร้อน, กรุงเทพมหานคร : สวิชาญการพิมพ์, 2538.

22. ดวงพร ทองงาม และ พรรณพิศ สุวรรณกูล. Systemic mycosis. จุฬาอายุรศาสตร์ 9 (2539) : 128-134.

23. Zeind, C.S., Cleveland, K.O., Menon, M., Brown, J.R., and Solomon, D.K. Cryptococcal meningitis in patients with the acquired immunodeficiency syndrom. Pharmacotherapy 14 (1996): 547-561.

24. Antimicrobial Agents: Antifungal Agents, in Molinoff, P. B., Rudden, R. W. (eds). The Pharmacological basic of therapeutics 9th ed, 1176-1184. St Louis New York : The McGraw-Hill companies, 1996.

25. Montaner, J. and Philips, P. AIDS related illness. in: McCormack, J. ed, Drug therapy decision making guide, 100-112. Philadelphia : W.B.Saunders company, 1996.

26. McEvoy, G.K. (ed.). AHFS Drug Information 97. 661-675. The American society of Health-System Pharmacists : Bethesda, 1997.

27. Daneshmend, T.K., and Warnock, D.W. Clinical Pharmacokinetics 8 (1983): 17-42.

28. Antimicrobial Agents: Drugs used in TB, MAC and Leprosy, in Molinoff, P. B., Rudden, R. W. (eds). The Pharmacological basic of therapeutics 9th ed, 1159-1167. St Lovis New York : The McGraw-Hill companies, 1996.

29. Debruyne, D., and Ryckelynck, J. P. Clinical Pharmacokinetics of Fluconazole. The Annals of Pharmacotherapy 24 (1993) : 10-27.

30. Jaruratanasirikul, S., and Kleepkaew, A. Lack of effect of fluconazole on pharmacokinetics of rifamicin in AIDS patients. The British Society for antimicrobrial Chemotherapy 38 (1996): 877-880.

31. Brammer, K. W., Farrow, P. R., and Faulkner, J. K. Pharmacokinetics and Tissue Penetration of Fluconazole in Humans. Reviews of Infectionus Diseases 12 (1990) : S318-S326.

32. Tett, S., Moore, S., and Ray, J. Phamacokinetics and Bioavailability of Fluconazole in Two Grops of Males with Human Immunodeficiency Virus (HIV) Infection Compared with Those in a Group of Males without HIV Infection. Antimicrobial Agents and Chemotherapy 39 (1995) : 1835-1841.

33. Tatro, D. S. Drug Interaction facts 2000. Missorri : A Wolters Kluwer Company, 2000.

34. Tucker, R. M., and et al. Interaction of Azoles with Rifampin, Phenytoin, and Carbamazepine: In Vitro and Clinical Observations. CID 14 (1992) : 165-174.

35. Nicolau, D. P., and et al. Rifampin-Fluconazole Interaction in critically ill patients. The Annals of Pharmacotherapy 29 (1995) : 994-996.

36. Koks, C. H. W., Meenhorst, P. L., Hillebrand, M. J. X., and Beijnen, J. H. Pharmacokinetics of Fluconazole in Salica and Plasma after Administration of an Oral Suspension and Capsules. Antimicrobial Agents and Chemotherapy 40 (1996) : 1935-1937.

37. สุเทพ จารุรัตนศิริกุล. ปฏิกริยาต่อกันของยาด้านเภสัชจลศาสตร์ในผู้ป่วยเอดส์. พิมพ์ครั้งที่ 1. กรุงเทพ : โอ เอส พริ้นติ้ง เฮาส์, 2542.

38. Fluconazole, in Lunn, G., and Schmuff N. (eds), HPLC Methods for Pharmace Analysis, 615-620. New York : A Wiley-Interscience Publication, 1997.

39. Walsh, T. J., and et al. Experimental Basis for Use of Fluconazole for Preventive or Early Treatment of Disseminated Candidiasis in Granulocytopenic Hosts. Reviews of Infectionus Diseases 12 (1990) : S307-S317.

40. Del-poeta, M., and et al. In-vitro activity of dicationic aromatic compounds and fluconazole against Cryptococcus neoformans and Candida spp. J Antimicrob Chemother 44 (1999): 223-8.

41. Aller, Al., and et al. Correlation of fluconazole MICs with clinical outcome in cryptococcal infection. J Antimicrob Chemother 44 (2000): 1544-8.

42. Aoki, F.H., and et al. The susceptibility of 23 strains of Cryptococcus neoformans isolated from CNS fluid of AIDS patients to seven antifungal drugs in Brazil. AIDS 12 (1998): 538.

43. Pfaller, M.A., and et al. In vitro activities of voriconazole, fluconazole, and itraconazole against 566 clinical isolates of Cryptococcus neoformans from the United States and Africa. Antimicrob Agents Chemother 43 (1999): 169 -71.

44. Jaruratanasirikul, S., and Kleepkaew, L.C. Lack of effect of fluconazole on the pharmacokinetics of rimfampicin in AIDS patients. J Antimicrob Chemother 38 (1996): 877-80.

45. Microbiology laboratory. Reported the range of MIC of 29 strains of *C. neoformans* isolated from CSF of AIDS patients. Microbiology laboratory. Bumrasnaradura hospital. Thailand.

APPENDICES

APPENDIX A

Patient Report Form (page1)

Patient Report Form page 1

Subject No.			Code ( )C , ( )E	
1. Admission Number	2. Hospital Number		Analyse	
3. Patient Name			4 ☐☐	5 ☐
4. Age	5. Sex (1) male			
6. Weight	(2) female		6 ☐☐	7 ☐☐
7. Occupation	8. Marital Status	(1) single		
9.Know HIV infect (years)		(2) married	8 ☐	11 ☐☐
10. Admission Date/Discharge Date		(3) widowed		
11. Stay in hospital (days)		(4) divorced	9 ☐☐	
12. Signs and Simptoms			121 ☐	122 ☐
(1) fever	(1) yes	(2) no		
(2) headache	(1) yes	(2) no	123 ☐	124 ☐
(3) nausea/vomiting	(1) yes	(2) no		
(4) neck stiffness	(1) yes	(2) no	125 ☐	126 ☐
(5) Cough	(1) yes	(2) no		
(6) Dyspnea	(1) yes	(2) no	127 ☐	128 ☐
(7) Impaired consciousness	(1) yes	(2) no		
(8) Seizure/ Convulsion	(1) yes	(2) no	129 ☐	121 ☐
(9) Papilledema	(1) yes	(2) no		
(10) Visual change	(1) yes	(2) no	1211 ☐	1212 ☐
(11) Photophobia	(1) yes	(2) no		
(12) Hearing loss	(1) yes	(2) no	1213 ☐	1214 ☐
(13) Skin lesions	(1) yes	(2) no		
(14) Lymphadenopathy	(1) yes	(2) no	1215 ☐	1216 ☐
(15) Hepatomegaly	(1) yes	(2) no		
(16) Splenomegaly	(1) yes	(2) no	131 ☐☐	132 ☐☐
13. Base line lab	14. Others Drug			
(1) Hct (%)			133 ☐☐	134 ☐☐
(2) WBC (10^3/mcl)				
(3) RBC (10^6/mcl)			135 ☐☐	136 ☐☐
(4) Hb (gm/dl)	(6) BUN (mg/dl)			
(5) Platelet count (10^3/mcl)	(7) Creatinine (mg/dl)		137 ☐☐	

Patient Report Form (page2)

Patient Report Form page 2

Subject No.			Code ( )C , ( )E				
14. CSF exam	base line	week 1	week 2	week 4	week 8	week 12	14.1 ☐☐
(1) OP/CP (<200 mm H_2O)							
(2) WBC (<10 cells/mm^3)							14.2 ☐☐
(3) Lymphocyte							
(4) PMN							14.3 ☐☐
(5) Monocyte							
(6) Protein (<45 mg/dl)							14.4 ☐☐
(7) Glucose (>40 mg/dl)							
(8) India ink							14.5 ☐☐
(9) Culture							
							14.6 ☐☐

15. Drug conc (mcg/ml)							
	time 0	time 1	time 2	time 4	time 8	time 12	time 24
phare 1							
phare 2							
phare 3							

14.7 ☐☐

14.8 ☐☐

14.9 ☐☐

16. Pharmacokinetic Parameters					
	half life	Ke	AUC	C_{max}	T_{max}
phare 1					
phare 2					
phare 3					

APPENDIX B

Detail data

Standard Curve

Concentration (mcg)	Ratio No.						Mean	SD	%CD
	No.1	No. 2	No.3	No.4	No.5	No.6			
1	0.415	0.402	0.392	0.358	0.290	0.379	0.373	0.045	12.100
3	0.488	0.482					0.485	0.004	
5	0.737	0.685	0.702	0.765			0.722	0.035	
10	1.023	1.140	1.153	1.031	1.155	1.220	1.120	0.078	6.926
15	1.530	1.541					1.536	0.008	
20	1.893	2.037	2.034	1.872	2.044	1.987	1.978	0.077	3.888
40	3.681	3.701	3.680	3.600	3.712	3.577	3.658	0.056	1.538

Detail data of demographic data

No.	Group	Age (years)	Sex	Weight (kg)	Occupation	Marital status	Rifam/dose	Hct (%)	WBC (x1000 cell/mm3)	BUN (mg/dL)	Cr (mg/dL)
1	FLU	46	male	no data	employee	married	0	35.00	5.80	20.00	0.71
3	FLU	32	male	48	employee	married	0	37.00	3.80	9.00	0.72
5	FLU	26	male	no data	government	single	0	39.00	4.90	14.50	0.90
6	FLU+RIM	31	male	50	employee	single	600	34.00	3.10	1.00	1.16
7	FLU	32	male	51	government	single	0	36.00	4.20	17.00	0.91
8	FLU	28	male	48	unemployed	single	0	27.70	2.46	12.40	1.21
9	FLU	37	male	no data	employee	married	0	24.00	4.63	17.70	0.90
11	FLU+RIM	42	male	48	comercial	single	600	27.00	12.60	8.00	0.60
12	FLU	32	female	38	employee	married	0	35.00	1.70	5.70	0.60
13	FLU	35	male	no data	employee	married	0	42.00	5.80	20.00	1.00
14	FLU+RIM	37	male	no data	employee	married	600	29.00	3.58	15.00	0.75
15	FLU	34	female	48	comercial	married	0	32.00	3.24	18.00	1.01
16	FLU	29	female	39	employee	single	0	26.00	7.11	17.90	1.48
17	FLU	24	female	45	employee	single	0	31.00	3.24	11.00	0.51
18	FLU+RIM	31	male	no data	employee	single	600	24.00	5.32	25.00	1.83
19	FLU+RIM	31	male	47	employee	single	600	32.00	4.68	37.00	2.33
20	FLU	31	female	38	employee	married	0	23.00	2.04	18.00	1.04
21	FLU	34	male	45	comercial	married	0	30.00	4.18	20.00	0.71
22	FLU	35	male	no data	comercial	married	0	25.00	4.07	20.00	1.01
23	FLU	42	female	40	unemployed	widwed	0	17.00	2.03	9.70	0.68
24	FLU+RIM	31	male	48	employee	married	600	36.00	5.20	13.00	0.90
25	FLU	30	male	no data	comercial	married	0	32.00	4.70	12.00	1.00
26	FLU+RIM	27	female	39	unemployed	single	450	24.00	2.63	10.00	0.86
27	FLU	27	male	48	employee	single	0	34.00	4.10	9.00	0.84
28	FLU+RIM	23	male	47	government	single	600	26.00	3.50	11.00	1.30
29	FLU+RIM	25	male	no data	comercial	single	600	28.00	5.10	16.00	0.90
30	FLU+RIM	30	female	39	comercial	married	600	39.00	4.36	19.00	0.70

Detail data of demographic data (continue)

No.	Group	Age (years)	Sex	Weight (kg)	Occupation	Marital status	Rifam/dose	Hct (%)	WBC (x1000 cell/mm3)	BUN (mg/dL)	Cr (mg/dL)
31	FLU+RIM	27	male	45	employee	single	600	24.00	3.20	16.00	1.10
32	FLU+RIM	33	male	no data	government	married	600	37.00	5.50	12.00	1.07
33	FLU+RIM	37	male	58	comercial	married	600	33.00	6.47	9.00	1.19
35	FLU	34	male	46	employee	married	0	40.00	3.90	8.00	1.13
36	FLU	32	male	no data	comercial	single	0	38.00	4.50	9.10	0.98
37	FLU	25	male	50	employee	single	0	40.00	3.80	7.00	1.08
38	FLU+RIM	21	male	49	comercial	married	600	38.80	4.20	7.00	0.81
39	FLU+RIM	23	female	no data	student	single	450	39.50	4.02	12.30	1.10
40	FLU+RIM	33	female	45	government	married	450	32.20	4.20	10.80	1.18
41	FLU+RIM	34	male	no data	employee	married	600	35.00	10.20	15.00	1.10
42	FLU+RIM	25	female	39	unemployed	married	450	33.60	5.32	10.80	0.81
43	FLU+RIM	48	male	no data	employee	married	600	37.00	5.80	11.00	1.06
44	FLU+RIM	39	female	no data	argiculturis	widwed	450	28.80	4.80	11.08	1.01

Note:

A group of patients who received only Fluconazole

A group of patients who concomitant administered Fluconazole with Rifampicin

Detail data of area ratio between fluconazole and internal standard

No.	Ratio of Fluconazole										
	Fluconazole 400 mg/day									200 mg/day	
	step 1 : day 8							step 2 : day 15		step 3 : day 8	
	0	1	2	4	8	12	24	0	4	0	4
3	1.302	1.593	1.671	1.956	1.689	1.490	1.312	1.332	1.954	0.768	1.055
5	1.241	1.536	1.617	1.500	1.335	1.301	1.121	1.135	1.500	0.703	0.904
6	1.027	1.324	1.643	1.508	1.389	1.230	1.054	1.079	1.579	0.699	0.973
7	1.290	1.565	1.787	1.869	1.700	1.590	1.333	1.300	1.857	0.761	1.000
8	1.207	1.332	1.603	1.794	1.655	1.521	1.290	1.202	1.658	0.758	1.003
9	1.299	1.535	1.800	1.968	1.800	1.699	1.399	1.362	1.876	0.850	1.127
11	1.110	1.212	1.730	1.527	1.390	1.254	1.099	1.121	1.654	0.660	0.922
12	1.701	2.023	2.641	2.507	2.365	2.188	1.779	1.766	2.499	1.137	1.530
13	1.251	1.446	1.678	1.859	1.699	1.593	1.296	1.283	1.826	0.839	1.113
14	1.015	1.450	1.620	1.581	1.368	1.224	1.086	1.121	1.599	0.666	0.909
15	1.321	1.532	1.775	1.931	1.781	1.628	1.369	1.348	1.898	0.848	1.135
16	1.485	1.644	1.877	2.142	1.961	1.855	1.529	1.496	2.010	1.029	1.356
18	0.987	1.217	1.447	1.350	1.213	1.081	0.900	0.895	1.369	0.587	0.848
19	0.826	1.015	1.394	1.566	1.410	1.227	0.962	0.959	1.565	0.633	0.910
20	1.445	1.845	2.081	1.952	1.701	1.628	1.357	1.293	1.912	1.001	1.357
22	1.295	1.495	1.779	1.727	1.563	1.456	1.262	1.210	1.637	0.822	1.076
23	1.291	1.584	1.734	1.999	1.804	1.659	1.381	1.327	1.900	0.901	1.199
24	0.864	1.114	1.237	1.370	1.205	1.114	0.845	0.884	1.384	0.531	0.754
26	1.282	1.688	1.910	1.995	1.900	1.666	1.245	1.113	1.832	0.721	1.039
28	1.111	1.247	1.627	1.502	1.416	1.253	1.061	1.103	1.556	0.689	0.953
29	0.925	1.058	1.433	1.632	1.439	1.286	1.112	1.128	1.754	0.716	1.006
30	1.008	1.203	1.441	1.599	1.403	1.269	1.006	1.005	1.588	0.697	0.990
31	1.112	1.586	1.791	1.719	1.552	1.399	1.061	1.100	1.710	0.689	1.003
33	1.303	1.568	1.863	2.265	1.975	1.696	1.381	1.355	2.258	0.810	1.205

Note: A group of patients who received only Fluconazole

A group of patients who concomitant administered Fluconazole with Rifampicin

Detail data of fluconazole concentrations

No	Fluconazole concentrations										
	Fluconazole 400 mg/day									200 mg/day	
	step 1 : day 8							step 2 : day 15		step 3 : day 8	
	0	1	2	4	8	12	24	0	4	0	4
3	12.085	15.524	16.451	19.818	16.663	14.303	12.206	12.440	19.802	5.759	9.160
5	11.357	14.856	15.815	14.425	12.477	12.072	9.936	10.102	14.430	4.998	7.373
6	8.831	12.348	16.116	14.527	13.118	11.237	9.144	9.439	15.361	4.950	8.192
7	11.940	15.199	17.818	18.788	16.793	15.490	12.454	12.059	18.646	5.679	8.515
8	10.959	12.440	15.644	17.901	16.257	14.679	11.944	10.904	16.295	5.649	8.549
9	12.047	14.846	17.975	19.969	17.975	16.779	13.231	12.798	18.872	6.734	10.007
11	9.813	11.014	17.146	14.744	13.121	11.517	9.678	9.946	16.252	4.484	7.588
12	16.802	20.618	27.929	26.347	24.669	22.568	17.728	17.578	26.252	10.125	14.778
13	11.485	13.783	16.538	18.670	16.787	15.530	12.008	11.864	18.289	6.608	9.844
14	8.690	13.831	15.849	15.391	12.869	11.155	9.524	9.938	15.600	4.551	7.430
15	12.311	14.807	17.676	19.530	17.757	15.947	12.873	12.626	19.131	6.709	10.112
16	14.254	16.125	18.886	22.019	19.880	18.622	14.767	14.375	20.460	8.852	12.727
18	8.355	11.072	13.794	12.647	11.030	9.464	7.324	7.270	12.876	3.621	6.716
19	6.450	8.686	13.175	15.208	13.366	11.199	8.058	8.020	15.194	4.166	7.441
20	13.778	18.510	21.301	19.773	16.807	15.946	12.740	11.981	19.299	8.520	12.730
22	11.995	14.370	17.729	17.112	15.169	13.911	11.613	10.991	16.051	6.405	9.411
23	11.956	15.415	17.199	20.328	18.028	16.304	13.013	12.382	19.155	7.341	10.862
24	6.902	9.857	11.310	12.892	10.938	9.858	6.677	7.135	13.053	2.962	5.598
26	11.843	16.652	19.279	20.279	19.162	16.394	11.412	9.844	18.354	5.209	8.974
28	9.827	11.427	15.930	14.451	13.437	11.502	9.231	9.724	15.088	4.827	7.953
29	7.624	9.194	13.631	15.991	13.699	11.897	9.830	10.022	17.428	5.153	8.576
30	8.602	10.909	13.724	15.604	13.275	11.692	8.576	8.569	15.466	4.920	8.388
31	9.839	15.440	17.869	17.020	15.043	13.228	9.233	9.692	16.911	4.828	8.546
33	12.101	15.227	18.724	23.482	20.044	16.744	13.013	12.705	23.396	6.265	10.934

Note: A group of patients who received only Fluconazole

A group of patients who concomitant administered Fluconazole with Rifampicin

Detail data of pharmacokinetic parameters

No.	Ke (hr^{-1})	Ka	Cmax (mg/L)	Tmax (hours)	Area Under Curve (mg.hr/L)	Half-Lift (hours)	Clearance (L/hr)	Vd (L)
3	0.014987	0.76458	17.803	3.9448	378.84	46.249	1.055855	70.45137
5	0.020847	2.3999	15.114	1.4565	294.97	33.25	1.35607	65.04869
6	0.027033	1.045	14.903	2.8608	284.32	25.641	1.406866	52.04252
7	0.021349	0.71896	18.258	3.8088	371.18	32.467	1.077644	50.47751
8	0.022782	0.46651	17.016	5.1497	351.21	30.426	1.13892	49.99209
9	0.022146	0.54877	19.212	4.6676	394.21	31.299	1.014688	45.8181
11	0.025743	0.84652	14.957	3.2617	291.61	26.926	1.371695	53.2842
12	0.022703	0.72938	26.714	3.803	537.34	30.531	0.744408	32.78895
13	0.024063	0.50048	17.888	4.7902	364.61	28.805	1.097063	45.59127
14	0.026339	1.3889	15.279	2.369	289.18	26.317	1.383222	52.5161
15	0.022568	0.55553	18.762	4.4971	383.72	30.714	1.042427	46.19048
16	0.021811	0.44576	20.91	5.1934	435.64	31.78	0.918189	42.09753
18	0.030752	1.0299	12.906	2.6702	239.08	22.54	1.67308	54.40557
19	0.036436	0.5112	14.394	4.7601	270.29	19.024	1.479892	40.6162
20	0.02376	1.3897	20.121	2.2417	389.26	29.173	1.027591	43.24877
22	0.020904	0.88587	16.925	3.162	341.88	33.158	1.170001	55.97021
23	0.023586	0.53835	19.186	4.6274	390.41	29.388	1.024564	43.43949
24	0.034548	0.59129	12.315	4.0129	231.22	20.063	1.729954	50.07393
26	0.0323	0.59126	20.415	4.0286	389.12	21.46	1.027961	31.8254
28	0.026414	0.76423	14.706	3.4128	286.95	26.241	1.393971	52.77395
29	0.027045	0.52228	14.741	4.9723	293.28	25.629	1.363884	50.43018
30	0.032418	0.54285	14.675	4.3109	281.52	21.382	1.420858	43.8293
31	0.032013	1.1165	17.503	2.6377	319.62	21.652	1.251486	39.09306
33	0.032672	0.47713	21.441	4.7866	415.28	21.216	0.963206	29.48107

Note: A group of patients who received only Fluconazole

A group of patients who concomitant administered Fluconazole with Rifampicin

Detail data of CSF analysis

No.	Week	OP (mm H2O)	WBC(cells/mm3)	Protein (mg/dL)	Glucose (mg/dL)	No.of fungus cell (cells/HPF)	culture
1	0	455	20	38.8	42	3	positive
	3	290	24	25	61.7	1	positive
	4	250	1	20.8	53.2	1	negative
3	0	640	0	50.4	52	16	positive
	3	390	1	35	50.8	5	positive
	4	255	0	38	55	1	negative
5	0	574	44	97.4	46	7	positive
	3	381	10	45	45	3	positive
	4	290	5	37.7	47	1	negative
6	0	398	71	55.47	17	50	positive
	1	264	17	127	50.1	25	positive
	4	241	10	45	55.3	10	negative
7	0	340	26	80.7	26.5	3	positive
	3	223	10	42.2	45.5	1	negative
8	0	421	65	44	49	5	positive
	3	340	30	40.5	45	3	positive
	4	189	9	32.6	42	1	negative
9	0	398	10	44	47	5	positive
	3	321	15	40.5	50.5	3	positive
	4	240	10	35.5	45.8	0	negative
11	0	487	15	26.7	45.5	3	positive
	3	274	10	35.8	42	3	positive
	4	221	10	40.4	59.5	1	negative
12	0	230	14	31	39	20	positive
	3	160	8	41	54	1	positive
	4	156	3	43	55.5	1	negative
13	0	420	10	52	48	10	positive
	3	223	11	50.7	55	0	negative
14	0	250	48	88	55	4	positive
	3	187	10	43	56	1	negative
15	0	398	1	22.4	50.8	8	positive
	3	275	5	25.8	45.5	5	positive
	4	125	1	40.2	50.5	3	negative
16	0	333	15	71	29	3	positive
	3	98	10	45	49	0	negative
17	0	440	1	10.3	48	1	positive
	3	240	1	25.6	49.5	1	negative
18	0	480	4	58.8	33.9	30	positive
	3	221	10	41.9	40	20	positive
	4	175	2	40	42	3	negative
19	0	390	26	64.2	28	3	positive
	3	250	6	51.2	42	1	negative
20	0	270	68	71.7	35	5	positive
	3	244	59	67.2	33	1	positive
	4	240	7	43.9	69	1	negative
21	0	482	59	58	40	20	positive
	3	350	50	45	45	5	positive
	4	150	10	44	68	1	negative
22	0	670	19	33	40.5	52	positive
	3	455	10	30	55	5	positive
	4	276	1	20.3	48	3	positive
	6	190	0	25	45	1	negative

No.	Week	OP (mm H2O)	WBC (cells/mm3)	Protein (mg/dL)	Glucose (mg/dL)	No.of fungus cell (cells/HPF)	culture
23	0	384	1	21	42	50	positive
	3	320	0	35.5	43.8	5	positive
	4	210	0	40.2	45.5	0	negative
24	0	256	10	37.6	47	7	positive
	3	140	7	30.1	58	3	negative
25	0	280	60	67.7	32	10	positive
	1	108	19	45.6	39	1	negative
26	0	340	48	32	46	3	positive
	3	176	3	35	55	3	negative
27	0	368	9	45.8	22	20	positive
	3	198	20	44	47	10	positive
	4	230	5	40	45	1	negative
28	0	450	55	76.7	49	20	positive
	1	280	25	58	45	5	positive
	2	180	1	45	50	1	negative
29	0	298	3	59.7	36	15	positive
	3	225	1	48.2	45.5	10	positive
	4	180	1	40.6	48.3	1	negative
30	0	400	7	30.4	55	10	positive
	3	350	12	41	53	4	positive
	4	220	5	34.74	58	1	negative
31	0	390	45	51	22	10	positive
	3	250	10	38.6	58.5	1	negative
32	0	480	9	29.2	53	25	positive
	3	342	8	38	55	15	positive
	4	200	2	45	48	3	negative
33	0	325	18	28	24	35	positive
	3	250	9	40	48	0	negative
35	0	465	38	68	48.8	20	positive
	3	381	10	48	49.5	10	positive
	4	189	10	42.5	45.5	0	negative
36	0	354	10	68	40	1	positive
	3	270	10	48	38.9	5	positive
	4	198	5	45	48	1	negative
37	0	365	0	42.8	35.5	20	positive
	3	235	1	43.2	45.6	5	negative
38	0	540	10	49.8	42.2	10	positive
	3	365	5	45.5	45.5	3	positive
	4	276	0	40.8	48.8	1	positive
	6	210	1	40.5	50.5	0	negative
39	0	368	10	48.8	56.5	10	positive
	3	156	0	42.8	45.5	3	negative
40	0	460	10	50.5	35.8	10	positive
	3	320	1	48.5	40.5	5	positive
	4	222	0	40.2	45.5	1	negative
41	0	480	4	59.8	38.9	25	positive
	3	279	2	45.9	42.3	15	positive
	4	165	1	38.8	40.8	2	negative
42	0	380	10	58	38.9	15	positive
	3	153	10	42	45	5	negative
43	0	487	50	38.8	40	35	positive
	3	354	25	40.2	42.2	20	positive
	4	208	5	55.5	45.5	5	negative
44	0	425	1	55.4	52	15	positive
	3	274	0	35.5	41	10	positive
	4	220	0	32.2	40.8	1	negative

Note: A group of patients who received only Fluconazole

A group of patients who concomitant administered Fluconazole with Rifampicin

VITA

Miss Nawarat Thanompuangseree was born on the 22th of July in 1975 at Udornthanee Hospital, Amphur Muang, Udornthanee. She graduated with Bachelor degree in Pharmaceutical Sciences in 1997 from Faculty of Pharmaceutical Sciences, Khonkhan University. She current position is a phamacist in Department of Pharmacy, Surathani Hospital, Surathani Thailand.