Pharmacokinetic Interaction between Ciprofloxacin and Itraconazole in Healthy Volunteers

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ชื่อวิทยานิพนธ์ ปฏิกิริยาต่อกันทางเภสัชจลนศาสตร์ระหว่างยาซิพโปรฟลอกซาซินกับไอทราโคนาโซลในอาสาสมัครสุขภาพปกติ

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บทคัดย่อ

ไอทราโคนาโซลเป็นยาต้านเชื้อราในกลุ่มไตรเอโซล ออกฤทธิ์ครอบคลุมเชื้อได้อย่างกว้างขวาง มีวิถีการแปรรูปผ่าน cytochrome P450 ส่วนซิพโปรฟลอกซาซินเป็นยาในกลุ่มฟลูออโรควิโนโลนที่มีฤทธิ์กว้างในการฆ่าเชื้อแบคทีเรีย และมีฤทธิ์ยับยั้งเอนไซม์ CYP3A4 วัตถุประสงค์ของการศึกษาคือ เพื่อศึกษาการเกิดปฏิกิริยาระหว่างยาซิพโปรฟลอกซาซินกับไอทราโคนาโซลในอาสาสมัครชายไทยสุขภาพปกติเมื่อได้รับประทานยาแบบต่อเนื่อง อาสาสมัครสุขภาพปกติจำนวน 10 รายเข้าร่วมในการศึกษานี้ ในระยะแรกอาสาสมัครจะได้รับไอทราโคนาโซลขนาด 200 มิลลิกรัมหรือซิพโปรฟลอกซาซินขนาด 500 มิลลิกรัมสองครั้งต่อวันเป็นเวลา 7 วัน เว้นระยะห่างของการให้ยาเป็นเวลา 2 สัปดาห์ ในระยะที่สองอาสาสมัครทุกรายจะได้รับไอทราโคนาโซลขนาด 200 มิลลิกรัมร่วมกับซิพโปรฟลอกซาซินขนาด 500 มิลลิกรัมวันละ 2 ครั้งเป็นเวลา 7 วัน ผลการศึกษาพบว่าซิพโปรฟลอกซาซินทำให้ความเข้มข้นสูงสุดของไอทราโคนาโซลในพลาสมาเพิ่มขึ้น 53.08% และพื้นที่ใต้กราฟระหว่างความเข้มข้นของยาและเวลาตั้งแต่เวลา 0-∞ ชั่วโมงเพิ่มขึ้น 82.47% อย่างมีนัยสำคัญ ตามลำดับ ค่าครึ่งชีวิตของไอทราโคนาโซลเพิ่มขึ้นและอัตราการกำจัดยานี้ลดลง แต่การเปลี่ยนแปลงดังกล่าวในทางตรงข้าม ไอทราโคนาโซลไม่มีผลต่อเภสัชจลนศาสตร์ของยาซิพโปรฟลอกซาซินอย่างมีนัยสำคัญ การศึกษาสรุปได้ว่าซิพโปรฟลอกซาซินมีฤทธิ์ยับยั้งวิถีการแปรรูปของไอทราโคนาโซลโดยน่าจะผ่านการยับยั้งเอนไซม์ CYP3A4 ดังนั้น ควรลดขนาดของไอทราโคนาโซลลงเมื่อให้ร่วมกับซิพโปรฟลอกซาซิน และควรติดตามผลการรักษาอย่างใกล้ชิด เพื่อลดปฏิกิริยาต่อกันทางเภสัชจลนศาสตร์และพิษจากยาไอทราโคนาโซล

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ABSTRACT

Itraconazole, a broad spectrum triazole antifungal agent, is metabolized via cytochrome P450 isozyme system. Ciprofloxacin, a fluoroquinolone compound, is a CYP3A4 inhibitor which has a broad spectrum bactericidal activity. The aim of this study was to investigate the drug interaction between ciprofloxacin and itraconazole following a multiple oral dose in healthy Thai male volunteers. Ten healthy subjects participated in the study. In phase 1, each volunteers ingested 200 mg of itraconazole or 500 mg ciprofloxacin alone twice daily for 7 days, with 2 weeks washout period. In phase 2, they orally received 200 mg of itraconazole in combination with 500 mg ciprofloxacin twice daily for 7 days. The results indicated that ciprofloxacin significantly increased C_{max} and $AUC_{0-\infty}$ of itraconazole by 53.08% and 82.47% respectively. Half-life of itraconazole was increased while its CL was decreased but these changes did not reach significant level. Conversely, itraconazole had no significant effect on the pharmacokinetics of ciprofloxacin. In conclusion, ciprofloxacin has an inhibition effect on the metabolism of itraconazole, mostly likely through CYP3A4 inhibition. Therefore, dosage of itraconazole should be reduced and therapeutic outcome be closely monitored during co-administration with ciprofloxacin in order to minimize the pharmacokinetic drug interaction and toxicity.

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LIST OF ABBREVIATIONS AND SYMBOLS

AUC = Area under the concentration-time curve

Bid = Bis in die or twice daily

BUN = Blood urea nitrogen

°C = Degree Celsius

C_{max} = Maximum plasma concentration

CL/f = Oral clearance

cm = Centimeter

μm = Micrometer

Cr = Creatinine

C.V = Coefficient of variation

etc. = Et cetera

g = Gram

mg = Milligram

μg = Microgram

ng = Nanogram

h = Hour

K_{ab} = Absorption rate constant

λ_z = Elimination rate constant

Kg = Kilogram

L = Liter

μL = Micro liter

min = Minute

MRT = Mean residence time

mL = Milliliter

MW = Molecular weight

P = P value

r = Correlation coefficient

SD = Standard deviation

LIST OF ABBREVIATIONS AND SYMBOLS (Continue)

SGOT = Serum glutamic-oxaloacetic transaminase

SGPT = Serum glutamic-pyruvic transaminase

S.E. = Standard error

sec = Second

$T_{½\ ab}$ = Absorption half-life

$T_{½\ el}$ = Elimination half-life

T_{max} = Time to maximal serum concentration

U/L = Unit per Liter

V_d /F = Volume of distribution

Vs = Versus

v/v/v = Volume by volume by volume

% = Percent

® = Tread name

∞ = Infinite

rpm = Round per minute

CHAPTER 1

INTRODUCTION

Viral, fungal, yeast and bacterial infections in humans are common and worldwide health problem. Million of people died because of infection every year, including opportunistic microorganism (bacteria, virus, or fungus) infections in immunocompromised patients. *Mycobacterium tuberculosis* is a widely spread infectious disease. The World Health Organization (WHO) has launched the global tuberculosis (TB) control in 1997. During 2001-2005, the average rate of new tuberculosis cases was 6% per year. However, in the year 2005-2006, the average rate was decreased in half (WHO, 2008). In Thailand, TB infection showed an increasing trend, including in the middle age persons. The mortality rate was about 1.4% of all deaths in Thailand. Moreover, in HIV patients the morbidity and mortality rates from TB infections were reported to be about 13-17% and 13-18%, respectively (Jittimanee *et al*., 2009).

Ciprofloxacin is a potent fluoroquinolone chemotherapeutic agent of the second-generation group of nalidixic acid derivatives commercially introduced in the 1980s. It is widely used both in human and veterinary medicine to treat infections caused by gram-negative and gram-positive bacteria including Enterobacteriaceae (especially enteropathogens such as *Escherichia coli*, *Samonella* spp. and *Shigella* spp.), *Neisseria* spp., *Moraxella catarrhalis*, *Pseudomonas aeruginosa* and *Haemophilus* spp; values of MIC_{90} range from 0.5 to 6 μg/mL (Davis *et al*., 1996; William and Petri, 2001). In addition, the US FDA (2000) recommended oral 500 mg ciprofloxacin twice a day for post-exposure inhalation anthrax and the drug should be given as soon as possible after exposure and should be administered for a total of 60 days.

Ciprofloxacin is a dibasic compound [pKa (1) = 6.09; pKa (2) = 8.62] which is very high solubility in water but this solubility has stronger temperature dependence (Barbosa *et al*., 2001; Melo *et al*., 2005). The primary mechanism of

action of ciprofloxacin and other fluoroquinolones are to rapidly inhibit DNA synthesis by promoting cleavage of bacterial DNA in the DNA-enzyme complexes of DNA gyrase and type IV topoisomerase (Oliphant and Green, 2002). Ciprofloxacin is primarily metabolized in the liver by the microsomal enzymes to four metabolites, and it also acts as an inhibitor of CYP1A2 and CYP3A4 in rats and human microsomes (Mclellan *et al*., 1996).

Azole compounds are used extensively for the treatment of cutaneous and invasive fungal infections. Itraconazole is the primary prophylaxis agent treating systemic fungal infectious in patients with advanced human immunodeficiency virus (HIV) infection (Chariyalertsak *et al*., 2002) and liver transplant recipients (Colby *et al*., 1999). In addition, it is a drug of choice for patients with nonmeningeal infections due to *Blastomyces dermatitidis, Histoplasma capsulatum, Paracoccidioides brasiliensis* and *Coccidioides immitis*, and is often the best choice for treatment of pseudallescheriasis, an infection not responding to amphotericin B therapy, as well as cutaneous and extracutaneous sporotrichosis, *Tinea coporis* and extensive *Tinea versicolor* (Bennett, 2001). Itraconazole is a potent CYP3A4 inhibitor and is primarily metabolized by CYP3A4. The primary mechanism of action of itraconazole and azoles, in general, is the inhibition of sterol 14-α demethylase, a microsomal cytochrome P450- dependent enzyme system (Pinjon *et al*., 2005). Itraconazole is also a weak base (pKa = 3.7) (Grant and Clissold, 1989) and the bioavailability of oral itraconazole is affected by gastric acidity (Beule, 1996).

In Thailand, TB and fungal infections have been recognized as major public health problem and are opportunistic infections (OIs) especially in HIV patients. Common OIs are TB of both pulmonary and extra-pulmonary, oral candidiastis and herpes zoster (Satasit, 2002). In addition, cryptococcosis and *Penicillium marneffei* infections were reported in approximately 20% and in 7% of patients with acquired immunodeficiency syndrome (AIDS) in northern Thailand (Chariyalertsak *et al*., 2002).

Bacterial and fungal infections are common diseases in immunocompromised or in immunosuppressive patients. Hence, the chance of ciprofloxacin and itraconazole co-administration in clinical practice is possible and may lead to ciprofloxacin-itraconazole interaction. Previous study reported that

itraconazole or ketoconazole significantly (p<0.05) increases maximum concentration (C_{max}), elimination half-life ($T_{1/2}$), mean residence time (MRT) and area under the concentration-time curve (AUC) of ciprofloxacin when administered in combination in mice. However, the time to reach maximum concentration (T_{max}) is not changed. The ciprofloxacin clearance is significantly reduced by both agents (Abou-Auda *et al*., 2008). To our knowledge, there is no report on the pharmacokinetic interaction between ciprofloxacin and itraconazole in human. Therefore, the purpose of this investigation is to study the pharmacokinetic interaction of a multiple oral dose of ciprofloxacin and itraconazole in Thai healthy male volunteers.

CHAPTER 2

LITERATURE REVIEWS

2.1 Ciprofloxacin

Ciprofloxacin is a quinolone carboxylic acid derivative that has broad spectrum bactericidal activity. Its structural is 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-[1-piperazinyl]-3-quinolinecarboxylic acid. As the anhydrous form, its molecular formula is $C_{17}H_{18}FN_3O_3$ and its molecular weight is 331.3. As the monohydrochloride form, which is very high solubility in water but this solubility has stronger temperature dependence (Melo *et al*., 2005). Its pKa has been reported to be 6.09 and 8.62 for the protonated and deprotonated amino group (Barbosa *et al*., 2001). It is a white to pale yellow crystalline powder and its chemical structure is shown in Fig 1 below.

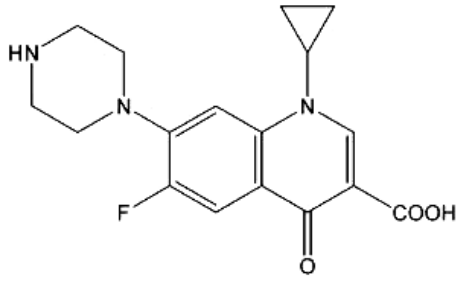


Figure 1. Chemical structure of ciprofloxacin.

Ciprofloxacin is a potent fluoroquinolone chemotherapeutic agent of the second-generation group of nalidixic acid derivatives commercially introduced in the 1980s. It is widely used both in human and veterinary medicine to treat infections caused particularly by gram-negative and some gram-positive bacteria (Davis *et al*., 1996). Ciprobay® is a film coated tablets available in 250 mg, 500 mg, 750 mg, 5% suspension and available in 250 mg and 2 mg/mL IV injection, respectively. The

inactive ingredients in tablet form are cornstarch, microcrystalline cellulose, silicon dioxide, crospovidone, magnesium stearate, hypromellose, titanium dioxide and polyethylene glycol.

2.1.1 Mechanism of action of ciprofloxacin

The mechanism of action of fluoroquinolones is similar to all quinolones. Ciprofloxacin inhibits DNA gyrase and topoisomerase IV (Hooper, 1999). DNA gyrase and topoisomerase IV both are essential for bacterial DNA replications. Topoisomerase II acts by breaking both strands of duplex DNA, passing another DNA strand through the break and resealing the initial broken strands (Roca, 1995). These activities are ATP dependent, with ATP hydrolysis serving to reset the enzyme for another cycle of strand passage. DNA gyrase, which is composed of two GyrA and two GyrB subunits, encoded by the *gyrA* and *gyrB* genes, respectively, is the only enzyme that introduces negative superhelical twists into bacterial DNA (Hooper, 1999).

Topoisomerase IV is composed of two subunits homologous to those of DNA gyrase. ParC (GrlA in *Staphylococcus aureus*), encoded by the *parC* gene, is homologous to GyrA and ParE (GrlB in S. *aureus*) encoded by the *parE* gene, is homologous to GyrB (Ferrero *et al*., 1994). Topoisomerase IV is the principal enzyme that decatenates or removes the interlinking of daughter chromosomes at the completion of a round of DNA replication and allows their segregation into daughter cells (Figure 2) (Zechiedrich and Cozzarelli, 1995; Hooper, 1999). Although these physiologic roles for topoisomerase IV and DNA gyrase have been determined largely from studies of *E. coli*, it is presumed that they will prove fundamentally similar in other bacteria.

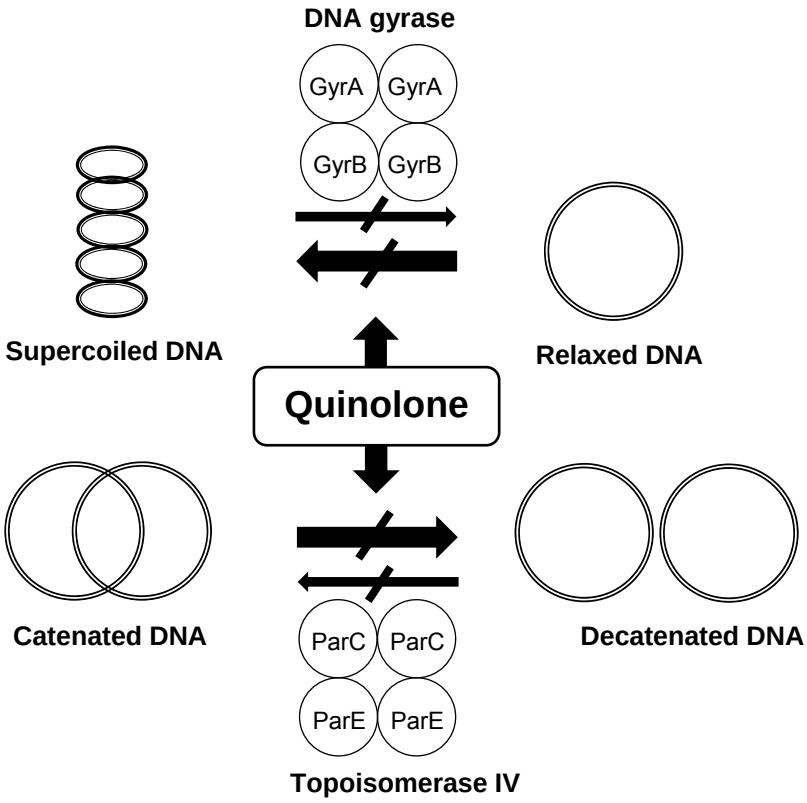


Figure 2. Major activities of DNA gyrase and topoisomerase IV. Quinolones block these activities by stabilizing an enzyme-DNA complex, which also functions as a barrier to the movement of other proteins such as DNA polymerase and RNA polymerase along the DNA (Hooper, 1999).

2.1.2 Pharmacokinetic of ciprofloxacin

Absorption: After oral administration in human, ciprofloxacin is rapidly absorbed from gastrointestinal tract into the systemic circulation (Vybiralova *et al*., 2005). The absolute bioavailability is ranging from 50-85% (Campoli-Richards *et al.*, 1988; von Rosenstiel and Adam, 1994). Maximam serum concentration (C_{max}; 0.8-3.9 mg/L) achieved 1 to 2 h after oral administration of single 250-270 mg doses. The intestinal absorption occurs mainly in the duodenum and the jejunum, mostly via passive-diffusion mechanisms (Harder *et al.*, 1990). The first-pass effect through the liver is thought to be unimportant (approximately 5%) (Vance-Bryan *et al.,* 1990).

Food does not impair the absorption of most quinolones. However, quinolone chelate with cation such as aluminium, magnesium, calcium, iron and zinc. These interactions significantly reduce absorption and bioavailability of this quinolones (Oliphant and Green, 2002).

Distribution: Ciprofloxacin diffuses into most body tissues (with particular affinities for the lungs and the prostate), with its protein binding approximately 30% (Campoli-Richards *et al.*, 1988; Vance-Bryan *et al*., 1990) and crosses physiological barriers by passive diffusion (Tsai and Wu, 2001). The AUC and T_{max} of 100 mg single dose of ciprofloxacin are 1.89 mg.h/L and 1.2-1.3 h both elderly and young volunteers (Hoffken *et al*., 1985), but the C_{max} in elderly patients was higher than young volunteers (Ball *et al*., 1986). In addition, sex- related difference pharmacokinetics of oral ciprofloxacin including C_{max}, oral clearance ($CL_{s/F}$) in females was greater than males, and the volume of distribution at steady state ($Vd_{ss/F}$) was smaller than males (Overholser *et al*., 2004). Ciprofloxacin has a large apparent volume of distribution (Vd; 2.1 to 5 L/kg after oral or intravenous administrations) and is concentrated in many body tissue and fluids, including bile, kidney, liver, gall bladder, prostate and lung tissue (Davis *et al*., 1996).

Metabolism: Fluoroquinolones is mainly metabolized in human and animals in the liver by formyl, methyl, sulfate, acetyl, oxidation and glucuronide group and breakdown of piperazine rings (Zeiler *et al*., 1987). Ciprofloxacin is also metabolized in the liver approximately 15-20% (Nouaille-Degorce *et al*., 1998) and four metabolites (desethyleneciprofloxacin (M1), sulfociprofloxacin (M2), oxociprofloxacin (M3) and formylciprofloxacin (M4)) are formed in human and mammals (Zeiler *et al*., 1987; Shah *et al*., 1996). However, CYPs pathway has not been studied with ciprofloxacin metabolism. In the fungus, *Pestalotiopsis guepini* have many metabolites such as N- acetylciprofloxacin (52%), desethyline-N-acetylciprofloxacin (9.2%), N- formylciprofloxacin (4.2%) and 7-amino-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (2.3%) (Parshikov *et al*., 2001a) which its metabolites may be possible to predicted pathway of the ciprofloxacin in human and animals metabolism (Parshikov *et al*., 2000; 2001a, b). Predominantly, four typical mammalian metabolites have been isolated from fungal cultures; all products show that the transformations occur at the piperazine ring

level. From their results, neither the fluorine atom nor the fluorinated ring is affected during metabolism (Murphy *et al*., 2009).

In many studies have demonstrated that ciprofloxacin is an inhibitor of CYP1A2 and CYP3A4 (Mclellan *et al*., 1996). The CYP1A2 is inhibited by reversible competitive in human and rat microsome (Sarker *et al*., 1990; Davis *et al*., 1995). However, it's inhibited by both a reversible noncompetitive and mechanism-base inhibitory in dogs (Regmi *et al*., 2005). Mclellan et al. (1996) have demonstrated that ciprofloxacin competitive reversible inhibitory effect on CYP3A activity in rat and human microsomes, but in dogs is lack of inhibition on CYP3A (Regmi *et al*., 2007). It has reported in patient successfully treated with methadone for more than 6 years and received oral ciprofloxacin (750 mg twice daily), which it was found that the patient become sedation, confusion and respiratory depression due to the inhibition of ciprofloxacin on CYP1A2 and CYP 3A4 activities (Herrlin *et al*., 2000).

Elimination: Ciprofloxacin is mainly eliminated via the kidney, both glomerular filtration and active tubular secretion appear to be implicated (Sörgel and Kinzig, 1993b). Nonrenal elimination is accounted for hepatic metabolism (15 to 20%), biliary secretion (less than 1%), and mostly intestinal secretion (10 to 15%) (Bergan, 1989; Parry *et al*., 1988; Sorgel and Kinzig, 1993a). The importance of intestinal elimination of ciprofloxacin has been confirmed in the rat using an *ex vivo* intestinal perfusion model as well as intestinal loops because this compound has an acidic as well as a basic group and its isoelectric point is at a pH of 7.4. In addition, in the blood, it is mainly found as a zwitterion which is one of the electrically neutral forms of molecule but with also behaves as an anionic and cationic form (Dautrey *et al*., 1999). Following intravenous administrations approximately 60% of dose excreted in the urine and 15% in the feces in unchanged form, approximately 2% excreted as M1 (4%), M2 (7%), M3 in the urine and the feces, M4 was detected in the urine in a few volunteers following an oral dose (Shah *et al*., 1996). In previous reviews approximately 94% of a radiolabeled 259 mg oral dose of ciprofloxacin was recovered in the urine and the feces with in 5 days of administration to healthy volunteers (Davis *et al*., 1996). The drug does not appear to be extensively metabolized, unchanged ciprofloxacin was major moiety recovered from both urine and feces approximately 40-60% of an administration dose.

The terminal half-life of ciprofloxacin ranged between 3 to 5 hour, high total and renal clearance (Davis *et al*., 1996). The $T_{1/2}$ of ciprofloxacin were not significant between healthy subjects and patients suffering from moderate renal failure (CL_{cr} = 30-45 mL/min/1.73 m^2) (Bergan, 1989). However, in severe renal failure ($CL_{cr} \leq$ 30 mL/min/1.73m^2) the $t_{1/2}$ of ciprofloxacin and their metabolites was prolonged (Shah *et al*., 1996).

2.1.3 US FDA labeled uses

a) Bacterial infectious disease
b) Chronic bacterial prostatitis
c) Febrile neutropenia
d) Gonococcal cervicitis, urethritis
e) Infection of bone
f) Infection of skin or subcutaneous tissue
g) Infectious diarrheal disease
h) Infectious disease of abdomen
i) Infectious disorder of joint
j) Inhalation anthrax
k) Lower respiratory tract infection
l) Nosocomial pneumonia
m) Sinusitis
n) Typhoid fever
o) Urinary tract infection

2.1.4 Therapeutic efficacy

Ciprofloxacin is a synthetic fluorinated 4-quinolone has a broad spectrum antimicrobial activity. Ciprofloxacin is effective in the treatment of a wide variety of infections, particularly those caused by gram-negative pathogens including complicated urinary tract infections (Davis *et al*., 1996). Also, it has been found to be effective in the treatment of bronchopulmonary diseases caused by *Pseudomonas aeruginosa* in patients with cystic fibrosis (Raeburn *et al*., 1987).

Respiratory Tract Infections

The pharmacokinetic and pharmacodynamic efficacy of intrapulmonary administration of ciprofloxacin for the treatment of respiratory infections caused by pathogenic microorganisms resisting sterilization systems of alveolar macrophages (AMs) was evaluated by comparison with an oral administration. These results indicate that intrapulmonary administration is more effective in delivering ciprofloxacin to AMs and lung epithelial lining fluid (ELF) compared with oral administration, in spite of a low dose and it avoids distribution of ciprofloxacin to the blood. The concentration of ciprofloxacin in AMs and ELF-time curve (AUC)/minimum inhibitory concentration of ciprofloxacin (MIC) ratio and the maximum concentration of ciprofloxacin in AMs and ELF (C_{max})/MIC ratio were markedly higher than the effective values. The present study indicates that intrapulmonary administration of ciprofloxacin is an effective technique for the treatment of respiratory infections (Chono *et al*., 2007).

Tuberculosis

The first-line (or essential) antituberculous drugs are the most active agents with proven clinical efficacy that form the core of initial standardized treatment regimens. These are isoniazid, rifampicin, pyrazinamide and ethambutol (WHO, 2008) and the guidelines suggest using fluoroquinolones as second-line drugs for treating drug resistant tuberculosis or as a substitute for first-line drugs in cases of intolerance. HIV co-infection, tuberculosis is the most common cause of death in HIV-positive adults in low-income and middle-income countries (Corbett, 2003). Only one trial stratified data by HIV status (Kennedy, 1996). It tested ciprofloxacin substituted into first line regimens for drug-sensitive tuberculosis and reported that the time to sputum culture conversion was longer in HIV-positive participants. There is an urgent need to determine the most appropriate anti tuberculous regimens for HIV-positive people and for further trials to explore the role of fluoroquinolones in these regimens. Guidelines suggest using fluoroquinolones as second-line drugs for treating drug resistant tuberculosis or as a substitute for first-line drugs in cases of intolerance (Ziganshina and Squire, 2008).

Urinary tract infections

Bladder infections are very common in otherwise healthy women and short-course antimicrobial treatment appears effective for many episodes of cystitis. So, this study the efficacy and safety of oral ciprofloxacin 100 mg twice daily, ofloxacin 200 mg twice daily, or trimethoprim/sulfamethoxazole 160/800 mg twice daily in 668 women with acute, uncomplicated, symptomatic lower urinary tract infection and the most commonly isolated pathogen was Escherichia coli (81%). That result shown the treatment with ciprofloxacin, ofloxacin and trimethoprim/ sulfamethoxazole had similar efficacy when given for 3 days to treat acute, symptomatic, uncomplicated lower urinary tract infection in women (McCarty *et a*l., 1999; Iravani *et al*., 1999).

Inhalation Anthrax

The recommended adult dose of ciprofloxacin for post-exposure inhalational anthrax is 500 mg given orally twice a day. The recommended pediatric dose of ciprofloxacin for post-exposure inhalational anthrax is 15 mg/kg given orally twice a day. The adult intravenous dose is 400 mg twice a day; the pediatric intravenous dose is 10 mg/kg twice a day. Treatment with ciprofloxacin should begin as soon as possible after exposure. The drug should be administered for a total of 60 days (US FDA, 2000).

2.1.5 Side effects

Most reports of side effects include: gastrointestinal tract (nausea or vomiting and diarrhea), central nerves system (fatigue, dizziness and headache), dermatological (rashes and pruritus) and photosensitivity. Oral ciprofloxacin recipients worldwide, the incidences of gastrointestinal tract, central nerves system and dermatological are approximately 3.4, 1.1 and 0.7%, respectively. Intravenous administration, the incidences of gastrointestinal effects are mainly nausea 4.8%, vomiting or diarrhea 2.3%, elevated liver enzymes value 1% and dermatological effects are mainly rash 2.5% and pruritus 0.8% (Davis *et al*., 1996).

2.1.6 Toxicological effects

Possible targets of quinolones toxicity include the kidney, eye, juvenile joint. Ciprofloxacin has been reported a low incidence of adverse effects related to gastrointestinal, skin, hepatic, central nerves system and cartilage defects (Hooper, 1985; Boomer, 2005).

Renal toxicity: Pathologic changes in the kidney have been observed in animal studies of the systemic tolerability of both oral and parenteral forms of ciprofloxacin. The histologic findings include inflammation within the tubular wall and presence of acicular crystals in the lumen of the renal tubules. However, the inflammatory changes are not the result of a primary toxic action but are a secondary reaction caused by precipitation of foreign material in the kidney (Schluter, 1987). In clinical practice, a crystalluria due to ciprofloxacin was recorded in two out of 63,000 patients as well as in a patient who developed obstructive uropathy due to massive ciprofloxacin crystal precipitation in the distal ureters and bladder, after a 24-day treatment at a dose of 500 mg twice daily (Fogazzi *et al*., 2006).

Ocular toxicity: The eye was a target of particular concern in our investigations because some quinolones have been shown to induce ocular damage in animals. Ciprofloxacin, like other quinolones, did enter the lens, but concentrations were not higher than those found in serum. There was no evidence that accumulated in lens tissue, no change in lens density during long term study in monkeys (Schluter, 1987).

Juvenile joint: Animal studies have confirmed that a number of quinolones damage juvenile weight-bearing joints, ciprofloxacin caused the least damage only 10% of male rats receiving 100 to 250 mg/kg and latter causing damage only 10% of male rats at a heighest dosage level (Schluter, 1987).

In addition, Gurbay et al. (2001) have been reported ciprofloxacin induces free radical production in a dose and time dependent. The mechanism of radical formation by ciprofloxacin might be a results of metabolization of this drug by cytochrome P450 and /or redox reactions.

2.2 Itraconazole

Itraconazole (Sporal®) is an azole antifungal agents, is the synthetic compounds by naturally occurring compound produced of *Streptomyces nodosus*. The azoles are classified as imidazoles (miconazole and ketoconazole) or triazoles (itraconazole and fluconazole) according to whether they contain two or three nitrogen atoms, respectively, in the five-membered azole ring. The chemical designation is cis-4[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(lH-1,2,4-triazol-l-ylmethyl)-1,3-dioxolan-4yl] methoxy] phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2-(1-methylpropyl)-3H-1,2,4 triazol -3-one (Cutsem *et al*., 1984). Its molecular formula is $C_{35}H_{38}Cl_2N_8O_4$ and the structural formula is shown in figure 3. Its molecular weight is 705.64.

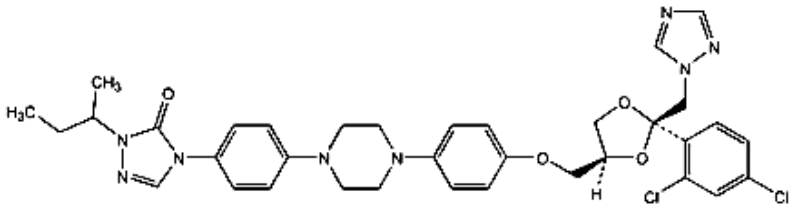


Figure 3. Structural formula of itraconazole

Itraconazole is a white to slightly yellowish powder. It is insoluble in water, very slightly soluble in dimethyl sulfoxide and alcohols and freely soluble in dichloromethane. Sporal® is a hard capsule available in 100 mg (blue cap and pink transparent body containing coated beads) and the inactive ingredients are corn starch, hypromellose, polyethylene glycol (PEG) 20,000 and sugar spheres.

2.2.1 The mechanism of action of itraconazole

The triazoles have a similar mechanism of action to that of the imidazole. Azole drugs target an enzyme of the ergosterol biosynthetic pathway known as lanosterol 14α-demethylase, which is encoded by the *ERG11* gene (Pinjon *et al*., 2005). The free azole nitrogen competes for oxygen at the catalytic heam iron

atom of cytochrome P-450 enzymes. Exposure of fungal cells to azoles causes inhibition of ergosterol synthesis and accumulation of 14α-methylated sterols, such as lanosterol and 14α-methyl-3,6-diol, which disrupt the structure of the membrane, alter its fluidity and the activity of membrane-bound enzymes and also accumulation of phospholipids and unsaturated fatty acids within fungal cells (Pinjon *et al.*, 2005). Itraconazole binds only weakly to mammalian cytochrome P-450 and it has much higher affinity than ketoconazole for fungal P-450 enzymes (Beule, 1996). The mechanism of action is shown in figure 4.

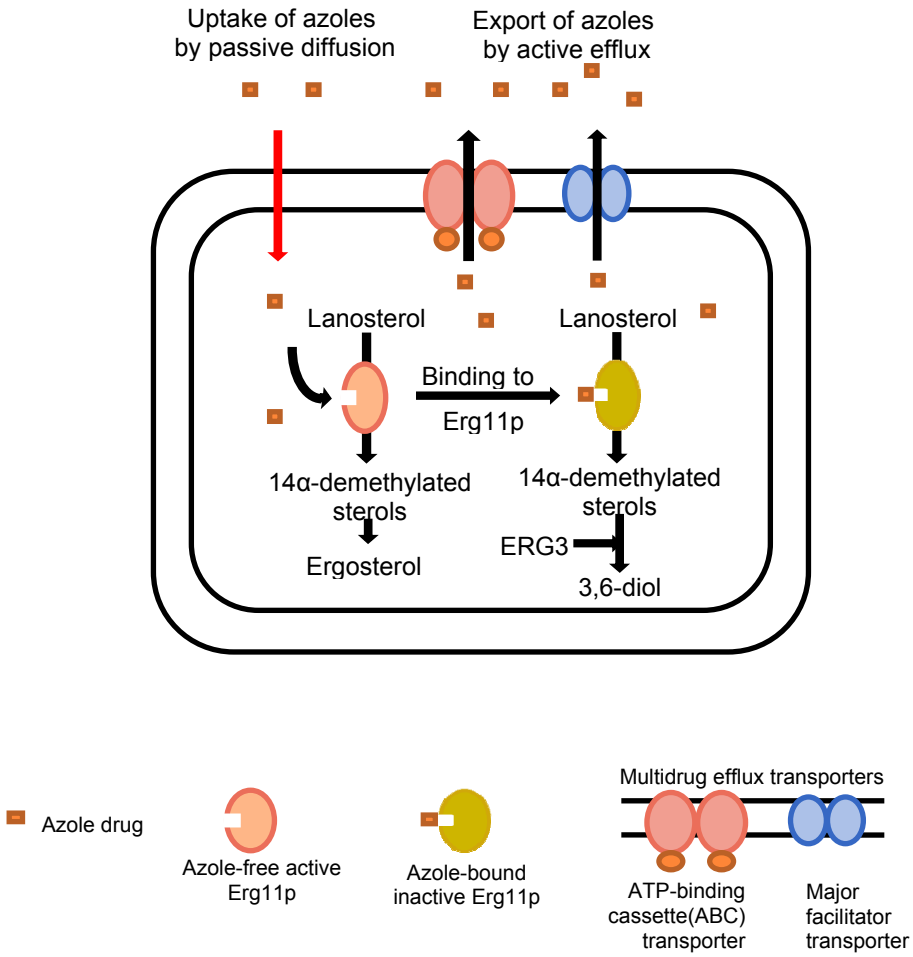


Figure 4. Model for mechanisms of action of itraconazole (adapted from Sanglard and Odds, 2002)

2.2.2 Pharmacokinetic of Itraconazole

The pharmacokinetic of itraconazole in human are characterized by good absorption, widely tissue distribution, with many tissue concentration was higher than those plasma, the elimination half-life of about 20-24 hours and transformation in to a large number of metabolites.

Absorption: Itraconazole is well absorbed. The absolute bioavailability of oral itraconazole is 55% and it was reduced by 40% when administrated under fasting conditions (Heykants *et al*., 1989). Itraconazole is only ionized at a low pH, such as in the gastric milieu and when administered with acidic beverage (pH 2.5), it's also a weak base (pKa = 3.7) (Grant and Clissold, 1989). In addition, the absorption of itraconazole was reduced when administered with omeprazole (Jaruratanasirikul and Sriwiriyajan, 1998). So, oral solution was developed, in which the drug is solubilised with the use of cyclodextrin (Willems *et al*., 2001). This bioavailability was higher than the capsule about 30 to 33% compared with a single 200 mg itraconazole (Barone *et al*., 1998) and the absorption was faster and better when taken without food (Van de Velde *et al*., 1996). However, the bioavailability of itraconazole oral solution and capsules in HIV-infected patients was reduced by approximately 20% and 50% compared to normal volunteers (Smith *et al*., 1991).

The plasma concentrations time curves of itraconazole in healthy volunteers were a wide inter individual and steady-state pharmacokinetics of 200 mg/day and 200 mg twice a day in healthy volunteers for 15 days. The T_{max} and C_{max} observed for single dosage was 3.0 hour and 412 ng/mL, respectively and for the twice a day was 6.0 hour and 1,980 ng/mL (Hardin *et al*., 1988). The absorption of itraconazole are ethnic dependence, such as the Japanese, the AUC and C_{max} was significantly reduced than in the German with both fed and fasting subjects (Yeates *et al*., 1995).

Distribution: Itraconazole is a highly protein bound (99.8%), primarily albumin, with only 0.2% available as free drug (Heykants *et al*., 1989), large volume of distribution (11 L/kg) (Templeton *et al*., 2008; Heykants *et al*., 1989) and accumulate in some tissue such as kidney, bone, muscle and liver in a large concentrations, while in the cerebrospinal fluid, urine and sputum contain at a low

concentrations. Tissue concentrations are 5 to 10 times higher than in plasma (Beule and Gestel, 2001).

Metabolism: Itraconazole is mainly metabolized primarily in the liver to a large number of metabolites (Grant and Clissold, 1989). Hydroxyitraconazole is a major metabolite accounting for less than 1-5% of the dose (Beule and Gestel, 2001). Itraconazole is metabolized by CYP3A4 sequentially to three metabolites (Figure 5) (Kunze *et al*., 2006), hydroxyitraconazole (OH-ITZ), keto-itraconazole (keto-ITZ) and N-desalkyl-itraconazole (ND-ITZ) which are all present in plasma following administration of itraconazole, because keto-ITZ and ND-ITZ are formed sequentially from itraconazole via OH-ITZ (Figure 6) (Isoherranen *et al*., 2004).

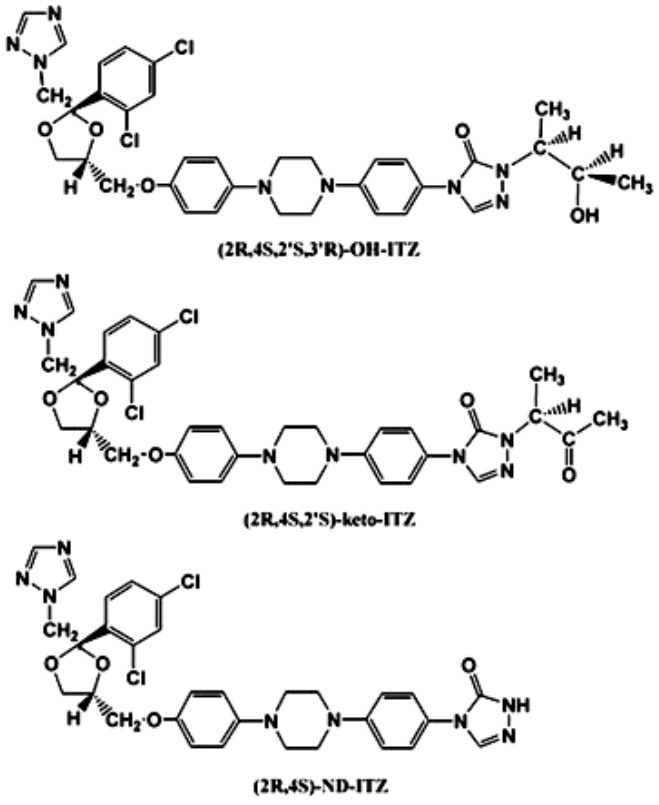


Figure 5. Structures of the metabolite of itraconazole

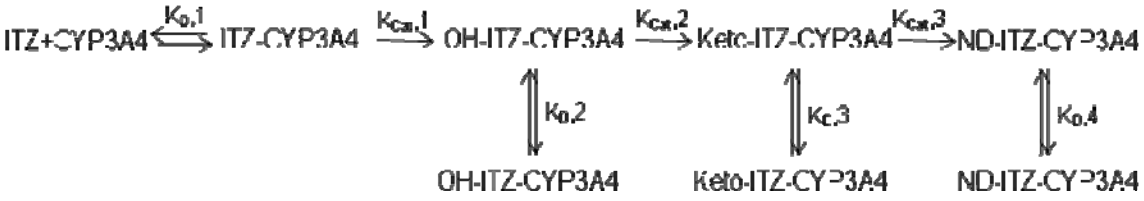


Figure 6. CYP3A4-catalased metabolic pathway for itraconazole

Itraconazole is a potent inhibitor of CYP450 including CYP3A4 and cause significant drug-drug interactions when combination with other CYP3A4 substrates (Bacman *et al*.,1998; Neuvonen *et al*., 1998; Mahnke *et al*., 2003). The metabolites are also potent inhibitors of CYP3A4 in *in vitro*, ND-ITZ is more potent inhibitor than OH-ITZ and parent compound in human microsome (Isoherranen *et al*., 2004).

Elimination: Elimination of itraconazole is biphasic, with a terminal half-life of approximately 20-24 h after a single dose (Beule and Gestel, 2001) and increase approximately 25-42 h after a multiple dose (Hardin *et al*., 1988). At the steady state, the terminal half-life was over to 30 hour, indicating that the itraconazole excretion mechanism is a saturated a clinical dose. Most metabolites are excreted through the bile approximately 54% of dose and approximately 35% of dose through the urine (Beule, 1996). Moreover, it is not excreted unchanged into the urine, only a small fraction (3-18%) is excreted unchanged in the feces (Poirer and Cheymol, 1998; Isoherranen *et al*., 2004). However, in rat and dogs have been excreted this metabolite through the urine and the feces (Heykant *et al.*, 1989).

2.2.3 Therapeutic efficacy

Itraconazole capsules dosage form are effective and indicated for the treatment of a number of localized and systemic fungal infections in adults, irrespective of their immune status (Haria *et al.*, 1996). These include vulvovaginal and oropharyngeal candidosis (Vladimir and Krcmery, 2005). Because of its lipophilicity, itraconazole distributes to the nails and the capsule formulation is effective in the treatment of onychomycosis. Itraconazole capsules can be used as

maintenance therapy in patients with AIDS and as prophylaxis before expected neutropenia, but as absorption is often impaired, blood monitoring should be performed and if necessary, the dose should be increased. There are inadequate data on itraconazole capsules in children (<12 years) and the elderly for their use to be recommended in these special patient populations.

Eruptive lichen planus (LP)

Itraconazole has been used to treat eruptive lichen planus (LP) in doses of 200 mg twice daily for 1 week each month for 3 months. Itraconazole was very effective in suppressing the progression of disease. It was also effective in producing resolution of lesions and symptomatic relief of pruritus (Khandpur *et al*., 2009).

Allergic bronchopulmonary aspergillosis (ABPA)

Allergic bronchopulmonary aspergillosis (ABPA) is a complex hypersensitivity reaction related to the presence of *Aspergillus fumigatus* colonizing the bronchial tree. The patients were followed during a 2-year reference period before the introduction of itraconazole and then during a 1-year itraconazole treatment period. These results shown that all patients successfully lowered oral glucocorticoid dose when received itraconazole. Due to the efficacy of itraconazole was reducing or eliminating the need for glucocorticoid therapy, along with clinical, biological and functional improvement (Salez *et al*., 1999).

Blastomycosis

Amphotericin B (AmB) is used in the treatment of patients who have severe blastomycosis and in CNS disease treatment with a lipid formulation of at a dosage of 3-5 mg/kg/day and followed by itraconazole 200 mg twice daily for 6-12 months and at least 1 year in CNS disease. For mild to moderate blastomycosis used 200 mg itraconazole once or twice daily for 6–12 months. Itraconazole is the preferred azole for initial therapy of patients with mild to moderate blastomycosis and as step-down therapy after treatment with AmB. When other azole agents are used, the medical record should document the specific reasons that itraconazole was not used and why other azoles were used. The drug levels should be measured during the first month in patients with disseminated or pulmonary blastomycosis and these levels should be documented in the medical record, as well as the physician's response to levels that are too low (Chapman *et al*., 2008).

Tinea corporis, pedis and capitis

A clinical response about 84-90% for *Tinea corporis* and *Tinea pedis* when treated with 200 mg daily for 1 week and 100 mg daily for 30 days or 200 mg for 15 days, respectively (Doncker *et al.,* 1997; Katsambas *et al*., 1993; Chow *et al*., 1998; De Doncker and Cauwenbergh, 1990). Itraconazole is the first azole derivative that matches griseofulvin for the treatment of tinea capitis in children. The drug also appears to be better tolerated than griseofulvin (Lopez-Gomez *et al*., 1994).

Toenails

The majority of patients included in clinical trials with itraconazole were affected by dermatophyte onychomycosis caused by Candida spp. such as *Candida onychomycosis* and *Candida paronychia* (Hay, 1994). There were reported that rates for toenail disease ranged from 67 to 77% (Gupta *et al*., 1998) and mycologic cure rate of about 74% when using 200 mg daily for 12 weeks (Doncker *et al.,* 1997) or 100 mg daily for 3–8 months (Hay *et al.*, 1988).

Gupta et al. (2009) reported that itraconazole was lower effective cure and safety than terbinafine therapy.

Haematological malignancies

The efficacy of oral itraconazole 200 mg capsules twice daily for prevention of systemic mycoses was investigated in granulocytopenic patients with haematological malignancies. The incidence of systemic yeast but not aspergillus infections was reduced by prophylaxis with itraconazole, but adequate comparability of patients groups is uncertain, superiority of other prophylactic managements, such as aerosolized amphotericin B in preventing aspergillus infections. When using itraconazole as antifungal prophylaxis, plasma concentrations should be obtained regularly to adapt the individual dosage especially in patients with unreliable intestinal absorption. In addition, based on our observations, a combination of itraconazole with vincristine should be avoided to prevent an increased incidence of vinca alkaloid-induced neurotoxicity (Bohme *et al*., 1996).

2.2.4 Side effects

The most common side effects of itraconazole compared with placebo are nausea and vomiting. Data on file of Amichai and Grunwald (1998) reported the side effects of itraconazole as follows:

Gastrointestinal side effects: Gastrointestinal disorders are the most common side effects in patients receiving itraconazole. Side effect are dose dependent, including a nausea (10.6%), vomiting (5.1%), diarrhea (3.3%), abdominal pain (1.5%), anorexia, dry mouth and constipation.

Cutaneous side effects: The most common cutaneous adverse reaction is eruption which appears in 4-8.6% in different reports. Eruption is more common in immunocompromised patients receiving immunosuppressive treatment. Pruritus was reported up to 2.5% of patients. Rarely, isolated reports of skin diseases related to itraconazole therapy have been described, including acute generalized exanthemic pustulosis, photo allergic reaction and urticaria.

Hepatic side effects: Elivation of serum liver enzymes, transaminases, and alkaline phosphatase, as well as serum bilirubin has been reported during itraconazole therapy, especially with the use of high dose and during prolong therapy. Although most of the hepatic side effects are dose dependent, idiosyncratic hepatic damage has also been reported and Tucker et al. (1990) reported the serum transaminases was increased about 5% of patients and 10.9% in invasive fungal infections (IFI) patients from Chaina (Aixia *et al*., 2006).

Hematologic side effects: Thrombocytopenia was reported in 0.5% of patients treated with itraconazole (Tuker *et al*., 1990).

Neurologic side effects: Headache (3.8%) and dizziness (1.7%) are the most common neurologic side effects and less common are fatigue and somnolence. Hallucinations and vertigo have also been reported (Tuker *et al*., 1990).

Cardiovascular side effects: Reversible edema of the extremities was reported in 0.4-3.5% of patients (Rosen, 1994) mild hypertension in several patient with high dose (600 mg/day). Isolated reports of Conn's syndrome (hypokalemia, edema, and hypertension) and ventricular fibrillation due to hypokalemia have also been described.

Endocrine side effects: In a reversible adrenal insufficiency have been reported during treatment with high doses (600 mg/day) of itraconazole. Gynecomastia has been described in 1% of patients tread with itraconazole 400 mg/day (Tuker *et al*., 1990).

Sexual dysfunction: A decrease in libido and impotence have been reported. Serum testosterone levels were normal in most patients complaining of impotence (Grant and Clissold, 1989).

Metabolic side effects: In a study by Tucker et al. (1990) hypokalemia was found in 6% and hypertriglyceridemia was reported in 9% of patients treated with high dose (400 mg/day) of itraconazole in Chaina.

2.3 Cytochrome P-450 System

Drugs are mainly metabolized by enzymes in the liver, intestine, lung, stomach and colon are found in the plasmic reticulum of cells in these tissues and are classified as microsomal enzymes. The cytochrome P450 (CYP) enzyme system consists of a superfamily of hemoproteins that catalyse the oxidative metabolism of a wide variety of exogenous chemicals including drugs, carcinogens, toxins and endogenous compounds such as steroids, fatty acids and prostaglandins (Shimada *et al*., 1994). There are 2 types of drug-metabolizing enzyme: phase 1, or mixed function oxidase and phase 2 metabolic reactions. Phase 1 reactions convert the parent hydrophobic drug to a more polar metabolite by oxidation, reduction or hydrolysis. These reactions expose or induce a functional group (-OH, $-NH_2$, -SH, or $–CO_2H$) and usually result in only a small increase in the hydrophilic of the drug. The CYP enzyme family plays an important role in phase-1 metabolism of many drugs. The broad range of drugs that undergo CYP mediated oxidative biotransformation is responsible for the large number of clinically significant drug interactions during multiple drug therapy (Badyal and Dadhich, 2001). Some examples of phase 1 reactions are presented in figure 7. These phase 1 metabolites are detoxified by phase 2 conjugation reactions with cellular macromolecule such as glucuronide, sulfate, acetate or an amino acid as shown in figure 8. Some examples of phase 2 reactions are presented in figure 9. Phase 2 preceding phase 1 reaction, although less common, can also occur. These reactions, either alone or in concert are responsible for the generation of readily excretable metabolites (Yan *et al*., 2001).

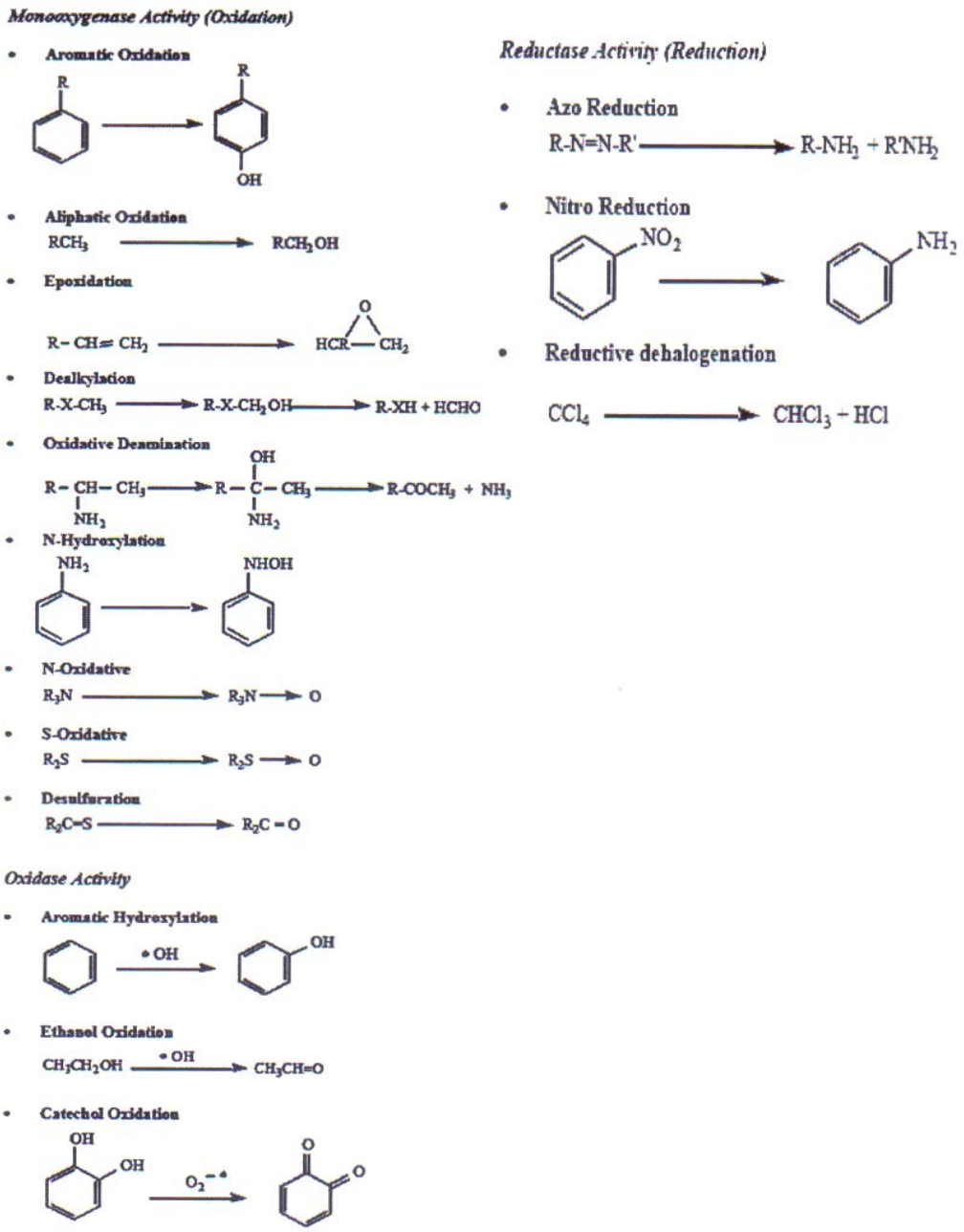


Figure 7. Examples of phase 1 reactions (Yan *et al*., 2001)

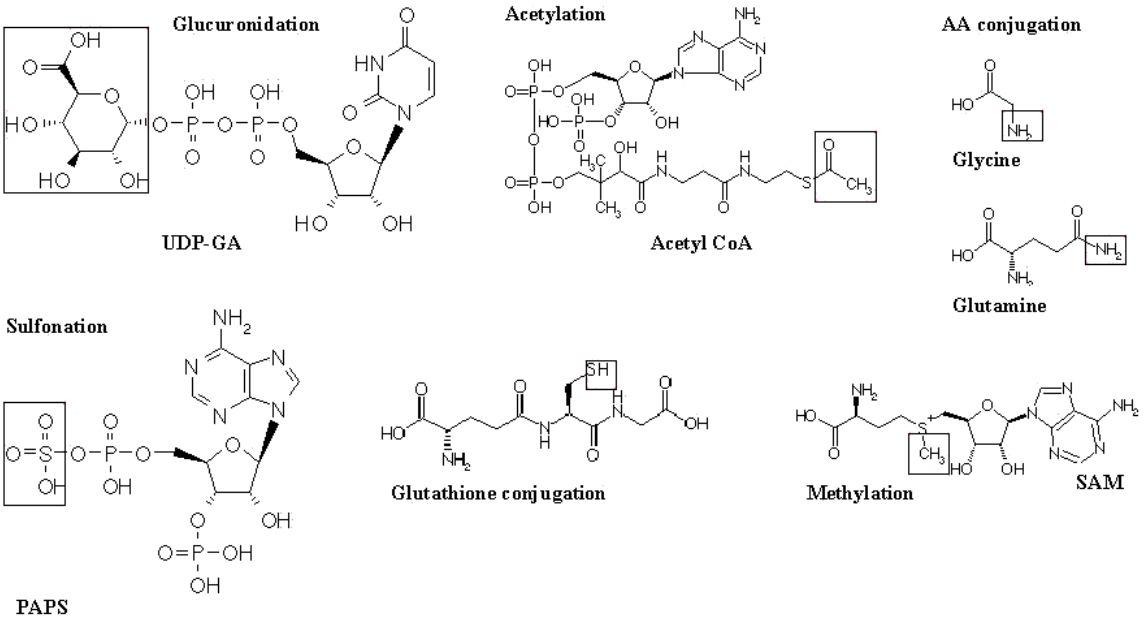


Figure 8. Structures of cofactors for phase 2 biotransformation (Jassal, 2004)

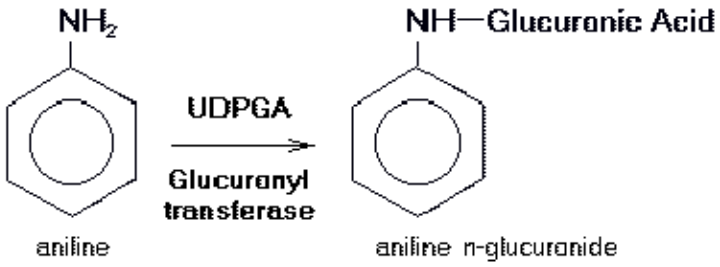


Figure 9. Reaction of glucuronide conjugation of aniline (Monosson, 2008)

Nomenclature of CYP

Nomenclature of CYP enzyme system has been established by CYP nomenclature committee (Nelson *et al*., 1996). The name cytochrome P450 is derived from the fact that these proteins have a haem group and an unusual spectrum. These enzymes are characterized by a maximum absorption wavelength of 450 nm in the reduced state in the presence of carbon monoxide. Naming a cytochrome P450 gene include root symbol "CYP" for humans ("Cyp" for mouse and Drosophila) an arabic numeral denoting the CYP family (e.g. CYP1, CYP2) letters A, B, C indicating subfamily (e.g. CYP3A, CYP3C) and another arabic numeral representing the individual gene/isoenzyme/isozyme/isoform (e.g. CYP3A4, CYP3A5) (Nelson *et al*., 1996). Of the 74 gene families so far described, 14 exist in all mammals. These 14 families comprise of 26 mammalian subfamilies (Nelson *et al*., 1996). Each isoenzyme of CYP is a specific gene product with characteristic substrate specificity. Isoenzymes in the same family must have >40% homology in their amino acid sequence and members of the same subfamily must have >55% homology (Levy, 1995). In the human liver there are at least 12 distinct CYP enzymes. At present it appears that from about 30 isozymes, only six isoenzymes from the families CYP1, 2 and 3 are involved in the hepatic metabolism of most of the drugs. These include CYP1A2, 3A4, 2C9, 2C19, 2D6 and 2E1 (Levy, 1995).

This CYP3A4 gene encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This protein localizes to the endoplasmic reticulum and its expression is induced by glucocorticoids and some pharmacological agents. This enzyme is involved in the metabolism of approximately half the drugs which are used today, including acetaminophen, codeine, cyclosporin A, diazepam and erythromycin. The enzyme also metabolizes some steroids and carcinogens. The members of the 3A subfamily are the most abundant CYP enzymes in the liver and account for about 30% of CYP proteins in the liver. High levels are also present in the small intestinal epithelium and thus make it a major contributor to presystemic elimination of orally administered drugs (Watkins *et al*., 1987). There is considerable inter-individual variability in hepatic and intestinal CYP3A activity (about 5-10 fold) (Shimada *et al*.,

1994). Since 40- 50% of drugs used in humans involve 3A mediated oxidation to some extent; the members of this subfamily are involved in many clinically important drug interactions (Thummel and Wilkinson, 1998). 3A4 is the major isoenzyme in the liver. 3A5 is present in the kidneys. Inducers of 3A4 usually do not upregulate 3A5 activity (Thummel and Wilkinson, 1998). CYP3A4 drug interactions are list in appendix H.

Mechanisms of Inhibition of CYP

The catalytic cycle of CYP consists of at least 7 discrete steps:

(i) binding of the substrate to the ferric form of the enzyme

(ii) reduction of the haem group from the ferric to the ferrous state by an electron provided by NADPH via CYP reductase

(iii) binding of molecular oxygen

(iv) transfer of a second electron from CYP reductase and/or cytochrome b5

(v) cleavage of the O-O bond

(vi) substrate oxygenation

(vii) product release (Figure 10)

Although impairment of any one of these steps can lead to inhibition of CYP enzyme activity, Step (i), (iii) and (iv) are particularly vulnerable to inhibition.

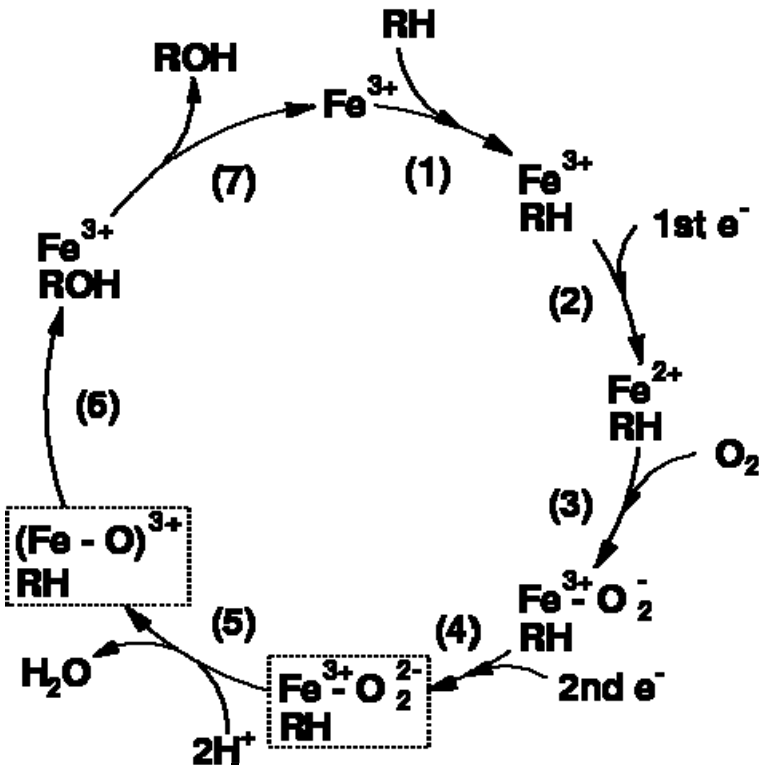


Figure 10. The catalytic cycle of cytochrome P450 (Shaik *et al*., 2004)

The mechanisms of CYP inhibition can be divided grossly into 3 categories:

1) Reversible Inhibition

Reversible inhibition is probably the most common mechanism responsible for the documented drug interactions. In mechanistic terms, reversible interactions arise as a result of competition at the CYP active site and probably involve only the first step of the CYP catalytic cycle. Many of the potent reversible CYP inhibitors are nitrogen-containing drug, including imidazoles, pyridines and quinolones. These compounds can not only bind to the prosthetic haem iron, but also to the lipophilic region of the protein. Inhibitors that simultaneously bind to both regions are inherently more potent inhibitors. The potency of an inhibitors is determined both by is lipophilicity and by the strength of the bond between its nitrogen lone electron pair and the prosthetic haem iron. For example, both

itraconazole and cimetidine are imidazole-containing compounds that interact with ferric CYP at its sixth axial ligand position to elicit a type 2 optical difference spectrum. The coordination of a strong ligand to the pentacoordinated iron, or the displacement of a weak ligand from the hexacoordinated haem by strong ligand, gives rise to a "type 2" binding spectrum. However, cimetidine is a relatively weak reversible inhibitor of CYP and apparent result of an intrinsic low binding affinity to microsomal CYP. This later property is most probably because of the low lipophilicity of cimetidine (log P = 0.4). On the other hand, itraconazole, potent CYP inhibitor, has a high lipophilicity (log P = 3.7). The quinolones are another class of nitrogen heterocycles that exhibit potent CYP inhibition. Ellipticine is a quinolone-containing compound that interacts with both ferrous and ferric CYP forms (Lin and Lu, 1998).

2) Quasi-Irriversible Inhibition via Metabolic Intermediate Complexation

A large number of drugs, including methylenedioxybenzenes, alkylamines, macrolide antibiotics and hydrazines undergo metabolic activation by CYP enzymes to form inhibitory metabolites. These metabolites can form stable complexes with the prosthetic haem of CYP, called metabolic intermediate (M1) complex, so that the CYP is sequestered in a functionally inactive state. M1 complexation can be reversed and the catalytic function of ferric CYP can be restored by *in vitro* incubation with highly lipophilic compounds that displace the metabolic intermediate from the active site. Other *in vitro* methods by which the ferrous complex can be disrupted include irradiation at 400-500 nm or oxidation to the ferric state by the addition of potassium ferricyanide. Dissociation or displacement of the M1 complex results in the reactivation of CYP functional activity. However, in *in vivo* situations, the M1 complex is so stable that the CYP involved in the complex is unavailable for drug metabolism and synthesis of new enzymes is the only means by which activity can be restored. The nature of the M1 complexation, therefore, considered to be quasi-irreversible (Lin and Lu, 1998).

3) Irreversible Inhibition of CYP

Drug containing certain functional groups can be oxidized by CYP to reactive intermediates that case irreversible inactivation of the enzyme prior to its release from the active site. The mechanism-based inactivation of CYP may result from irreversible alteration of haem or protein, or a combination of both. In general, modification of the haem group in variable inactivates the CYP, whereas protein alteration will result in loss of catalytic activity only if essential amino acids, which are vital for substrate binding, electron transfer and oxygen activations are modified.

Drugs containing terminal double-bond (olefins) or triple-bond (acetylenes) can oxidized by CYP to radical intermediates that alkylate the prosthetic haem group and inactivated the enzyme. The evidence for haem alkylation includes the demonstration of equimolar loss of enzyme and haem, as well as the isolation the structural and characterization of the haem adducts. Haem alkylation is initiated by the addition of activated oxygen to the internal carbon of the double or triple bond and is terminated by binding to haem pyrrole nitrogen.

The best known example of inactivation of CYP through protein modification by a suicide inactivator is that of chloramphenical. The dichloroacetamido group is oxidized to an oxamyl moity that acylates a lysine residue in the CYP active center. This acylation event interferes with the transfer of electrons from CYP reductase to the haem group of the CYP and thereby prevents catalytic turnover of the enzyme (Lin and Lu, 1998).

Mechanism of Induction of CYP

The induction of cytochrome P450 (CYP) enzymes by xenobiotics is a major concern due to the enhanced metabolism of pharmaceutical drugs and endogenous substrates. The oxidative metabolism of drugs to more polar metabolites is an important mechanism allowing for the elimination of xenobiotics (Tompkins and Wallace, 2007). In drug therapy, there are 2 major concerns related to CYP induction. First, induction will result in a reduction of pharmacological effects caused by increased drug metabolism. Secondly, induction may create an undesirable imbalance between “toxification” and “detoxification”. Like a double-edged sword, induction of drug metabolizing enzymes may lead to a decrease in toxicity through acceleration of

detoxification, or to an increase in toxicity caused by increased information of reaction metabolites. Depending upon the delicate balance between detoxification and activation, induction can be a beneficial or harmful response (Lin and Lu, 1998).

The mechanisms by which CYPs are induced have been an area of extensive research for many years. Many of the CYPs are induced in humans including CYP1A, CYP2A, CYP2B, CYP2C, CYP2E1, and CYP3A by a diverse array of compounds including drugs, industrial chemicals, natural products and ethanol. The inducible CYPs make up a large percentage of the CYPs in the human liver and are responsible for the metabolism of a large proportion of pharmaceutical drugs. In most cases, induction of CYPs occurs by a process involving de novo RNA and protein synthesis that has been demonstrated in studies using transcription and translation inhibitors. The induction of many CYPs occurs by a similar mechanism, where ligand activation of key receptor transcription factors including pregnane X receptor (PXR), constitutive androstane receptor (CAR), aryl hydrocarbon receptor (AhR) and others, leads to increased transcription. The induction of CYPs is highly conserved and is found not only in humans but also in many other species including rodent models. An alternative mechanism of CYP induction involves compounds that stabilize translation or inhibit the protein degradation pathway (Tompkins and Wallace, 2007).

In the human liver, CYP3A4 is the most highly expressed CYP enzyme and is highly inducible by a wide variety of xenobiotics. The mechanisms by which structurally diverse xenobiotics induce CYP3A4 protein expression have been extensively investigated. Induction of CYP3A4 can also have serious toxicological consequences as a result of increased drug metabolism that contributes to drug–drug interactions, the bioactivation of xenobiotics to carcinogenic or toxic metabolites and possibly endocrine disruption. The CYP3A4 induction by xenobiotics is now thought to be largely due to xenobiotic binding and activation of this orphan receptor. The Evans group named this protein the steroid and xenobiotic receptor (SXR) for its ability to bind such a wide variety of steroids and xenobiotics.

PXR is a member of the nuclear receptor superfamily and contains modular functional domains, including a DNA-binding domain and a ligand-binding domain. PXR is most closely related to the vitamin D receptor (VDR) and like VDR,

binds to DNA elements as a heterodimer with a retinoid X receptor alpha (RXRα). PXR response elements have been well characterized, with the receptor binding to direct repeat with a 3-nucleotide (nt) spacer (DR3), everted repeat with a 6-nt spacer (ER6) and a direct repeat with a 4-nt spacer (DR4). These elements have been identified in the human CYP3A4 and rat CYP3A23 promoters and sites are bound by PXR in the presence of xenobiotics. X-ray crystallography of the ligand-binding domain of PXR determined that PXR has a much larger ligandbinding pocket when compared to other receptors; enabling PXR to bind such a wide variety of ligands. Since the initial identification of PXR, a growing list of compounds has been found to bind and activate this receptor including the chemotherapeutic agents, environmental contaminants and antibiotics (Tompkins and Wallace, 2007).

The promiscuous nature of PXR binding to so many xenobiotics led Blumberg and Evans to propose that PXR acts as a nonspecific xenobiotic-sensor. The CYP3A magnitude of induction also varies greatly between individuals in human studies and it has been suggested that polymorphisms in PXR may be important in this wide variability (Tompkins and Wallace, 2007).

2.4 Transporters

Membrane transporters have long been recognized to be an importance class of proteins for regulating cellular and physiologic solute and fluid balance (Ho and Kim, 2005). With the initial sequencing of the human genome, it has been estimated that approximately 500 to 1200 genes encode transport proteins (Venter *et al*., 2001; Lander *et al*., 2001). A number of those transporters appear to facilitate or in some cases prevent drug passage through membrane barriers. These drug transporter proteins tend to be multi functional and often have normal physiologic role in terms of transporting endogenous substances such as sugar, lipids, amino acids, bile acids, steroids and hormones (Ho and Kim, 2005).

Drug transporter

Drug transporter are expressed in many tissue such as the epithelial cells of the intestine and kidney, hepatocytes, and brain capillary endothelial cells (figure 11) (Inui *et al*., 2000; Aubel *et al*., 2000; Mizuno *et al*., 2003), play key roles in the absorption, distribution and excretion of drugs and are one of the determinant factors governing drug disposition. Transporters have been classified as primary, secondary or tertiary active transporters. Secondary or tertiary active transporters are driven by an exchange of intracellular ions while the driving force for primary transporters, like ATP-binding cassette transporters, is ATP hydrolysis. The tissue distribution and elimination route of some drugs is determined by the degree of expression of each transporter subtype in each tissue and it corresponding substrate affinity (Mizuno and Sugiyama, 2002). Drug transporter can be generally separated in to 2 major classes- uptake and efflux transporters.

- Uptake transporters act by facilitating the translocation of drug into cells. Included with in this class of transporters are members of the organic anion transporting polypeptide (OATP, *SLCO*) family, organic anion transporter (OAT, *SLC22A*) family, organic cation transporter (OCT, *SLC22A*) family, organic cation/carnitine transporter (OCTN, *SLC22A*) family and peptide transporter (PEPT, *SLC15A*) family (Ho and Kim, 2005).

- Efflux transporters function to export drugs from the intracellular to the extra cellular milieu, often against height concentration gradients. Most efflux transporters are the member of the adenosine triphosphate (ATP)- binding cassette (ABC) super family of trans membrane proteins, which use energy derived from ATP hydrolysis to mediate substrate translocation across biologic membranes (Ho and Kim, 2005).

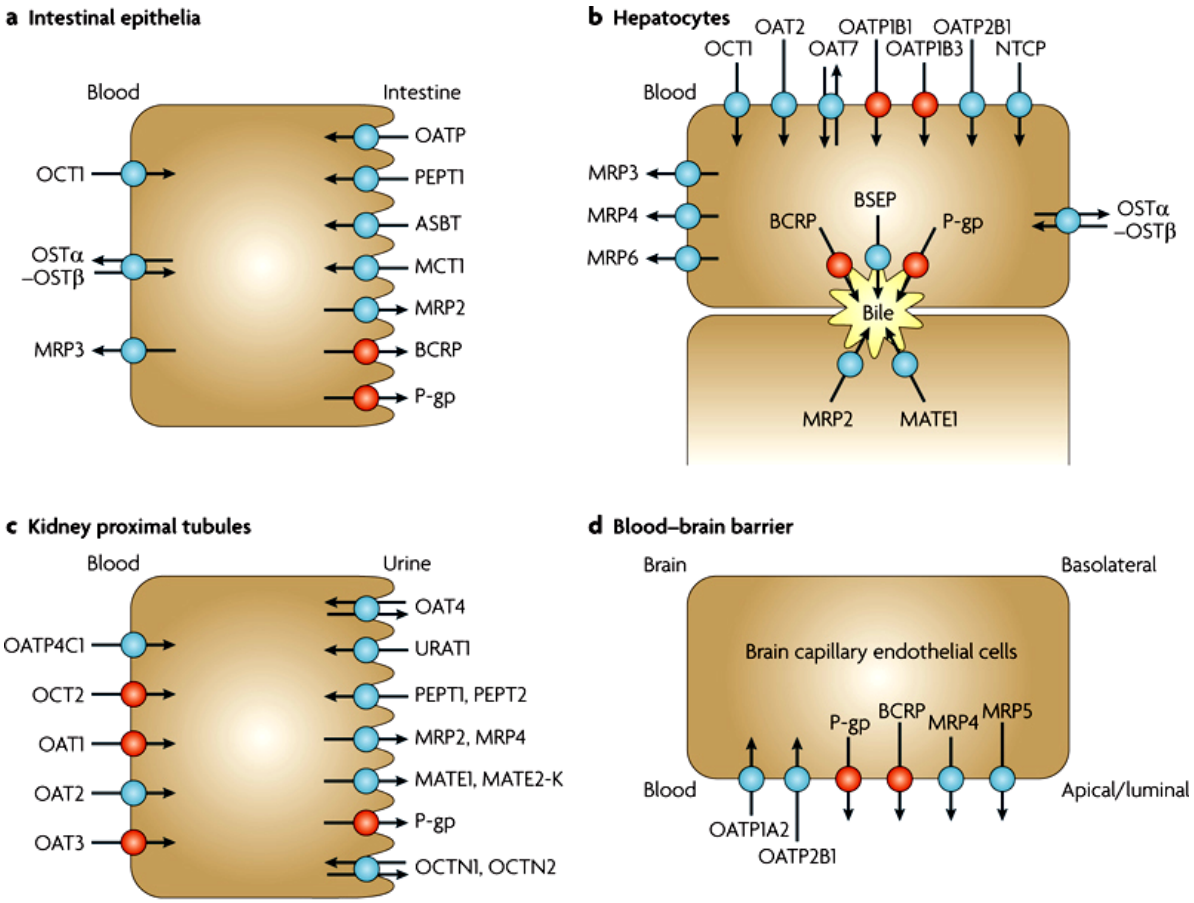


Figure 11. Major drug transporters in human (Giacomini *et al.*, 2010)

Transporters and tissue drug distribution

Targeted locolization of transporters appears to facilitate the intestinal absorption and urinary or biliary excretion of many drugs. In the small intestine, enterocytes possess a number of transporters critical for absorption of dietary constituents and drugs. In the liver, efficient extraction of drug from portal blood into hepatocytes is often madiated by uptake transporters expressed on the sinusoidal (basolateral) membrane and efflux transporters localized on the canalicular (apical) membrane of the hepatocyte are transport of drugs from portal circulation in to bile. In kidney, the coordinate function of uptake and efflux transporters localized to basolateral and apical membranes of proximal tubular cells. In blood brain barrier (BBB), localized to the luminal side of BBB endothelial cells (Ho and Kim, 2005).

Transporters and drug-drug interactions

As the number of newly approved drugs increase, medication errors and drug-drug interactions have become a significant source of patient morbidity and death. Factors such as age, diet, nonprescription drugs, herbal and alternative therapies may also influence the development and extent of drug interactions. In additions, inhibition of drug metabolizing enzymes, as well as transporters, is now recognized as the likely mechanism accounting for many drug-drug interactions, because transporters and CYP enzymes often share overlapping tissue expression and substrate capacities (Ho and Kim, 2005).

ABC transporter (ATP -binding cassette (ABC))

Several ABC transporter family members such as MDR1/p-gp (ABCB1) MDR3 (ABCB4), MRP1 (ABCC1), MRP2/CMOAT (ABCC2), MRP3 (ABCC3), MRP4/CMOATB (ABCC4), MRP5 (ABCC5), MRP6/ARA (ABCC6) breast cancer resistance protein and BCRP/ABCP (ABCG2) are expressed in the healthy human intestine. BCRP and MRP2 are more extensively expressed than MDR1 (Tsuji, 2002). The transcript levels of these nine ABC transporters in healthy human jejunum and in Caco-2 cells were correlated well, although BCRP exhibited a 100 fold lower transcript level in Caco-2 cells compared with jejunum (Taipalensuu *et al*., 2001).

P-glycoprotein or MDR1

P-gp/MDR1 is primary active transporters, encode by MDR1 or MRP transporters are responsible for the cellular extrusion of many kinds of drugs. P-gp transporter is a wide variety of lipophilic, structurally diverse drugs, such as vinca alkaloids and anthracyclines (Mizuno *et al.*, 2003). In the small testine, MDR1 (P-gp) has been determined to be a particularly importance efflux transporter that can actively extrude or pump drugs back into the intestinal lumen, effectively limiting the extent (bioavailability) of substrate drug absorption (Ho and Kim, 2005). The key role of MDR1 to drug absorption has been exemplified by studies in knockout mice with disruption of the homologous *mdr1'a* gene (Schinkel *et al*., 1994) such as the oral bioavailability of digoxin (Schinkel *et al*., 1995) and HIV1 protease inhibitors (Kim *et al*., 1998) is markedly increase in *mdr1'a* knockout mice in comparison with wide-type mice, indicating that MDR1 mediated drug efflux by enterocytes limit bioavailability of many drugs (Ho and Kim, 2005). In humans the intestinal MDR1 expression and activity has been shown to influence drug levels after administration of cyclosporine (INN, cyclosporine) (Lown *et al*., 1997) digoxin (Drecher *et al.*, 2003), further supporting its role in determining the bioavailability of commonly administered drug.

Structure of P-glycoprotein

MDR1 is a large gene spanning more thane 100 kb on chromosome 7, with 28 exons that are spiced in to 4.5 kb mRNA. The encoded P-gp is an integral membrane protein with a molecular weight of approximately 170 kDa was shown in figure12. P-gp function is an energy-dependent membrane pump, which extrudes generally cationic or neutral, hydrophobic drugs from cells.

In the liver, efflux transporters localized on the canalicular (Apical) membrane of the hepatocyte, such as MDR1, MRP2 and BCRP represent the final step in the vectorial transport of drugs from portal circulation in to bile (Ho and Kim, 2005). In addition to MDR1 a transporter previously referred to as the canalicular multispecific organic anion transporter, now referred to as MRP2, is responsible for the biliary excretion of numerous endogenous organic anion including bilirubin glucuronides, as well as drugs such as methotrexate (Masuda *et al*., 1997).

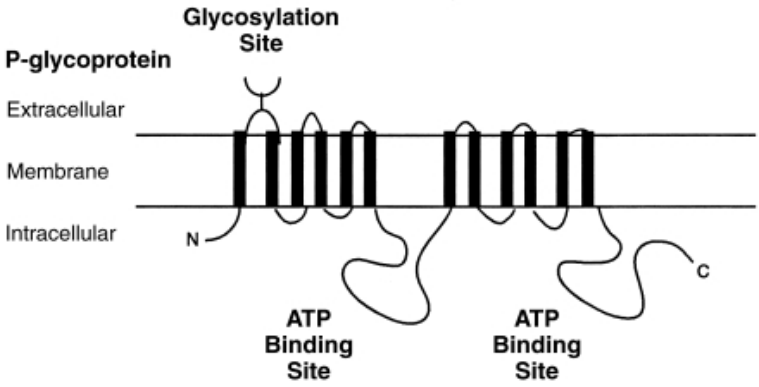


Figure 12. Structure of P-glycoprotein (P-gp) (Morrow and Cowan, 2000)

In the kidney, drug secretion also represents the coordinate function of uptake and efflux transporters localized to the basolateral and apical membranes of proximal tubular cells (Ho and Kim, 2005). Renal efflux transporters have not been as extensively studied, but members of the MRP and OATP families of transporters have been localized to the apical membrane of proximal tubular cells and likely contribute to the urinary elimination of substrate drugs (Ho and Kim, 2005). In the brain, the efflux transporters such as MDR1 localized to the luminal side of the BBB endothelial cells, prevent CNS entry of substrate drugs (Cardon-Cardo *et al.*, 1989). The importance of MDR1 expression at the level of the BBB has been shown in previous studies with *mdr1a* knockout mice (Schinkel *et al*., 1994). However, *mdr1a* knockout mice have been shown to be 50-100 folds more sensitive to the neurotoxic pesticide ivermectin and accumulation of this drug in the brain tissue of *mdr1a* (-/-) mice was noted to be 80-100 folds greater when compared with control mice (Schinkel *et al*., 1994).

MRP family

The multidrug resistance-associated proteins, or MRPs, are members of the ABCC subfamily of the ABC transporter superfamily (conjugate transporter-2 or CT2; 3.A.1.208). MRPs 1, 2, 3, 6 and 7 have a predicted secondary structure that contains 17 TMDs. MRPs 4 and 5 are smaller owing to the absence of TMDs 1–5. The MRPs that have been functionally characterized share the ability to support

primary active (ATP-dependent) export of a broad array of anionic substrates from cells (Wright and Dantzler, 2004).

- MRP1 (*ABCC1*) is broadly expressed in many cells types, with the highest expression generally found in the basolateral membrane of epithelial cells.

- MRP2 (*ABCC2*) was cloned, there was physiological evidence for the presence of an ATP-dependent transporter in the canilicular (apical) membrane of hepatocytes and has been shown to be expressed in the apical membrane of both human and rat, renal proximal tubule cells liver and intestinal cells.

- MRP3 (*ABCC3*) is expressed in the kidney, its principal site of expression in the rat and human is the intestine. In the human kidney, MRP3 is expressed in the basolateral membranes of distal convoluted tubule cells, but not in proximal convoluted tubule cells.

- MRP4 (*ABCC4*) is expressed in the apical membrane of human renal proximal tubule cells, which immediately implicates it in the secretion of selected OAs.

This 4 transports a broad range of anionic conjugates, including GSH, glucuronate and phosphate conjugates, the quantitative aspects of MRP4 selectivity differ rather markedly from those of MRP1, MRP2 and MRP3. MRP5 supports the transport of a wide array of anionic conjugates, including cAMP and cGMP, located in the kidney, however, not yet established. MRP6 is localized to the basolateral membrane of proximal tubule cells the function has been difficult to establish. Although the physiological action of MRP6 is not clear, it has been suggested that it may play a housekeeping role (Wright and Dantzler, 2004; Mizuno *et al*., 2002).

ATP-binding cassette, sub-family B member 11 (BSEP)

BSEP is a protein which in humans is encoded by the *ABCB11* gene or sPgp (sister of P-glycoprotein). ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, ABCG/White) (Dean, 2002). The BSEP protein is a member of the MDR/TAP subfamily (Strautnieks *et al*., 1998; Noe´ *et al*., 2002; Stieger *et al*., 2007; Dean, 2002) located on the canalicular membrane, mediates the transport of bile acids such as taurocholic acid. Intrahepatic cholestasis can be induced by interference with the secretion of biliary constituents resulting in an

intracellular accumulation of bile salts and other toxic bile constituents within hepatocytes (Mizuno *et al*., 2003).

Organic cation and anion transporter (OCT/OAT)

Renal secretion of organic electrolytes of broadly diverse chemical structures plays a critical role in limiting the body's exposure to toxic compounds of exogenous and endogenous origin (including a wide array of compounds of clinical importance). Because the physiological, pharmacological and toxicological importance of the renal secretion of "organic cations" (including bases; collectively, OCs) and "organic anions" (including acids; collectively, OAs). Schematic models of renal OC and OA secretion, based on the available functional evidence, typically showed a single peritubular "avenue of entry" into cells for each chemical class of substrate (OC or OA) and one (or two) luminal "avenue(s) of exit." These functionally characterized pathways were often (and continue to be) referred to as the "classical" pathways for renal secretion of organic cations and anions (Wright and Dantzler, 2004; Giacomini and Sugiyama, 2006).

Organic cation transport

Structurally diverse organic cations are secreted in the proximal tubule (Wright and Dantzler, 2004). Many secreted organic cations are endogenous compounds (e.g., choline, N-methylnicotinamide and dopamine) and renal secretion appears to be important in eliminating excess concentrations of these substances. However, a primary function of organic cation secretion is ridding the body of xenobiotics, including many positively charged drugs and their metabolites (e.g., cimetidine, ranitidine, metformin, procainamide and N-acetylprocainamide) and toxins from the environment (e.g., nicotine). Organic cations that are secreted by the kidney may be either hydrophobic or hydrophilic (Wright and Dantzler, 2004; Giacomini and Sugiyama, 2006).

Organic cations appear to cross the basolateral membrane by three distinct transporters in the SLC family 22 (*SCL22*): OCT1 (*SLC22A1*), OCT2 (*SLC22A2*) and OCT3 (*SLC22A3*). Organic cations are transported across this membrane down their electrochemical gradient (–70 mV). Transport of organic cations from cell to tubular lumen across the apical membrane occurs via an

electroneutral proton–organic cation exchange mechanism in a variety of species, including human, dog, rabbit and cat. Transporters assigned to the apical membrane are in the SLC22 family and termed novel organic cation transporters (OCTNs). In humans, these include OCNT1 (*SLC22A4*) and OCTN2 (*SLC22A5*). These bifunctional transporters are involved not only in organic cation secretion but also in carnitine reabsorption. In the reuptake mode, the transporters function as Na+ cotransporters, relying on the inwardly driven Na+ gradient created by Na^+, K^+-ATPase to move carnitine from tubular lumen to cell. In the secretory mode, the transporters appear to function as proton–organic cation exchangers. That is, protons move from tubular lumen to cell interior in exchange for organic cations, which move from cytosol to tubular lumen. The inwardly directed proton gradient (from tubular lumen to cytosol) is maintained by transporters in the SLC9 family (NHEs), which are Na+/H+ exchangers (antiporters). The bifunctional mechanism of OCTN1 and OCTN2 may not totally explain the organic cation–proton exchange mechanism that has been described in many studies in isolated plasma membrane vesicles. Of the two steps involved in secretory transport, transport across the luminal membrane appears to be rate-limiting (Giacomini and Sugiyama, 2006).

A number of charged amino acids are also conserved and their potential role in the function of these organic electrolyte transporters is discussed in upcoming sections.

- OCT1 (*SLC22A1*) was cloned from a cDNA library prepared from rat kidney. Orthologs were subsequently cloned from three additional mammalian species (mouse, rabbit and human**)** displaying from 95 to 78% identity with rOCT1. In addition, related sequences have also been cloned from *C. elegans* and *Drosophila melanogaster*. The mammalian isoforms vary in length from 554 to 556 amino acids (Wright and Dantzler, 2004) and have 12 putative transmembrane domains and include several N-linked glycosylation sites. A long extracellular loop between transmembrane domains 1 and 2 is characteristic of the OCTs. The gene for the human OCT1 is mapped to chromosome 6 (6q26). There are four splice variants in human tissues, one of which is functionally active, OCT1G/L554. In humans, OCT1 is expressed primarily in the liver, with some expression in heart, intestine and skeletal muscle. In mouse and rat, OCT1 is also abundant in the kidney, whereas in

humans, very modest levels of OCT1 mRNA transcripts are detected in kidney. The transport mechanism of OCT1 is electrogenic and saturable for transport of model small-molecular-weight organic cations including tetraethylammonium (TEA) and dopamine. Interestingly, OCT1 also can operate as an exchanger, mediating organic cation–organic cation exchange. That is loading cells with organic cations such as unlabeled TEA can trans-stimulate the inward flux of organic cations such as MPP^+. It also should be noted that organic cations can transinhibit OCT1. In particular, the hydrophobic organic cations quinine and quinidine, which are poor substrates of OCT1, when present on the cytosolic side of a membrane, can inhibit (*trans* inhibit) influx of organic cations via OCT1 (Giacomini and Sugiyama, 2006).

- OCT2 (*SLC22A2*) was isolated originally from a rat renal library, human, mouse, pig and rabbit. The initial rOCT2 clone had an open reading frame that coded for a 593-amino acid protein. The human, mouse, pig and rabbit orthologs, in contrast are 555, 553, 554 and 554 amino acids in length, respectively (Wright and Dantzler, 2004). Similar to OCT, OCT2 is predicted to have 12 transmembrane domains, including one N-linked glycosylation site. OCT2 is located adjacent to OCT1 on chromosome 6 (6q26). A single splice variant of human OCT2, termed OCT2-A, has been identified in human kidney. OCT2-A, which is a truncated form of OCT2, appears to have a lower K_m (or greater affinity) for substrates than OCT2, although a lower affinity has been observed for some inhibitors. Human, mouse and rat orthologs of OCT2 is expressed in abundance in human kidney and to some extent in neuronal tissue such as choroid plexus. In the kidney, OCT2 is localized to the proximal tubule and to distal tubules and collecting ducts. In the proximal tubule, OCT2 is restricted to the basolateral membrane. OCT2 mammalian species orthologs are greater than 80% identical, OCT2 is also present inneuronal tissues. However, studies with monoamine neurotransmitters demonstrate that dopamine, serotonin, histamine and norepinephrine have low affinities for OCT2 whereas OCT1 and OCT2 paralogs are approximately 70% identical. The transport mechanism of OCT2 is similar to that of OCT1 (Giacomini and Sugiyama, 2006).

- OCT3 (*SLC22A3*) was cloned from rat placenta. Human and mouse orthologs have also been cloned. OCT3 consists of 551 amino acids and is predicted to have 12 transmembrane domains, including three N-linked glycosylation sites.

hOCT3 is located in tandem with OCT1 and OCT2 on chromosome 6. Tissue distribution studies suggest that human OCT3 is expressed in liver, kidney, intestine and placenta although it appears to be expressed in considerably less abundance than OCT2 in the kidney. Like OCT1 and OCT2, OCT3 appears to support electrogenic potential-sensitive organic cation transport. Although the specificity of OCT3 is similar to that of OCT1 and OCT2 it appears to have quantitative differences in its affinities for many organic cations. Some studies have suggested that OCT3 is the extraneuronal monoamine transporter based on its substrate specificity and potency of interaction with monoamine neurotransmitters. Because of its relatively low abundance in the kidney, OCT3 may play only a limited role in renal drug elimination (Giacomini and Sugiyama, 2006).

- OCTN1 (*SLC22A4*) cloned originally from human fetal liver, is expressed in the adult kidney, trachea and bone marrow. The functional characteristics of OCTN1 suggest that it operates as an organic cation–proton exchanger. OCTN1-mediated influx of model organic cations is enhanced at alkaline pH, whereas efflux is increased by an inwardly directed proton gradient. OCTN1 contains a nucleotide-binding sequence motif and transport of its substrates appears to be stimulated by cellular ATP content. OCTN1 also can function as an organic cation–organic cation exchanger. Although the subcellular localization of OCTN1 has not been demonstrated clearly, available data collectively suggest that OCTN1 functions as a bidirectional pH and ATP-dependent transporter at the apical membrane in renal tubular epithelial cells (Giacomini and Sugiyama, 2006).

- OCTN2 (*SLC22A5*) was first cloned from human kidney and determined to be the transporter responsible for systemic carnitine deficiency. Rat OCTN2 mRNA is expressed predominantly in the cortex, with very little expression in the medulla and is localized to the apical membrane of the proximal tubule. OCTN2 is a bifunctional transporter. That is, it transports L-carnitine with high affinity in an Na^+ dependent manner, whereas, Na^+ does not influence OCTN2-mediated transport of organic cations such as TEA. Thus, OCTN2 is thought to function as both an Na^+ dependent carnitine transporter and an Na+-independent organic cation transporter. Similar to OCTN1, OCTN2 transport of organic cations is sensitive to pH, suggesting that it may function as an organic cation exchanger.

Therefore, transport of L-carnitine by OCTN2 is an Na^+ dependent electrogenic process (Giacomini and Sugiyama, 2006).

Organic anion transport

As with OCs, the proximal tubule is the primary site of renal OA secretion, as determined by studies employing stop flow, micropuncture and microperfusion. Substrates for the pathways involved in renal OA transport include a diverse array of weak acids that have a net negative charge on carboxylate or sulfonyl residues at physiological pH. Although a number of endogenous OAs have been shown to be actively secreted by the proximal tubule (e.g., 5′-hydroxyindoleacetic acid, riboflavin), it is generally accepted that the principal function of this process is clearing the body of xenobiotic agents, including many of the products of phase 1 and phase 2 hepatic biotransformation, as well as anionic drugs of therapeutic or recreational use (Wright and Dantzler, 2004). Two primary transporters on the basolateral membrane mediate the flux of organic anions from interstitial fluid to tubule cell: OAT1 (*SLC22A6*) and OAT3 (*SLC22A8*). Energetically, hydrophilic organic anions are transported across the basolateral membrane against an electrochemical gradient in exchange with intracellular α-ketoglutarate, which moves down its concentration gradient from cytosol to blood. The outwardly directed gradient of α-ketoglutarate is maintained at least in part by a basolateral Na^+ dicarboxylate transporter (NaDC3). The Na^+ gradient that drives NaDC3 is maintained by Na^+, K^+-ATPase (Giacomini and Sugiyama, 2006).

The mechanism responsible for the apical membrane transport of organic anions from tubule cell cytosol to tubular lumen remains controversial. Some studies suggest that OAT4 may serve as the luminal membrane transporter for organic anions. However, recent studies show that the movement of substrates via this transporter can be driven by exchange with α-ketoglutarate, suggesting that OAT4 may function in the reabsorptive, rather than secretory, flux of organic anions. The human ortholog of OATV1 is NPT1, or NaPi-1, originally cloned as a phosphate transporter. NPT1 can support the low-affinity transport of hydrophilic organic anions such as PAH. Other transporters that may play a role in transport across the apical membrane include MRP2 and MRP4, multidrug-resistance transporters in the ATP binding cassette family C (ABCC). Both transporters interact with some organic

anions and may actively pump their substrates from tubule cell cytosol to tubular lumen (Giacomini and Sugiyama, 2006).

- OAT1 (*SLC22A6*) was cloned from rat kidney. This transporter is greater than 30% identical to OCTs in the SLC22 family. Mouse, human, pig and rabbit orthologs have been cloned and are approximately 80% identical to human OAT1. The mammalian isoforms of OAT1 is very in length from 545 to 551 amino acids with features similar to those shown in figure 14. The gene for the human OAT1 is mapped to chromosome 11 and is found in an SLC22 cluster that includes OAT3 and OAT4. There are four splice variants in human tissues, termed OAT1-1, OAT1-2, OAT1-3 and OAT1-4. OAT1-2, which includes a 13-amino-acid deletion, transports PAH at a rate comparable with OAT1-1. These two splice variants use the alternative 5′-splice sites in exon 9. OAT1-3 and OAT1-4, which result from a 132-bp (44-amino-acid) deletion near the carboxyl terminus of OAT1, do not transport PAH. In humans, rat and mouse OAT1 is expressed primarily in the kidney, with some expression in brain and skeletal muscle (Giacomini and Sugiyama, 2006).

- OAT2 (*SLC22A7*) was cloned first from rat liver. This transporter has a gender-based tissue distribution between the liver and the kidney in rodents but not in humans, OAT2 is present in both kidney and liver. In the kidney, the transporter is localized to the basolateral membrane of the proximal tubule. Efforts to stimulate organic anion–organic anion exchange via OAT2 have not been successful, leading to the speculation that OAT2 is a basolateral membrane transporter that serves in the reabsorptive flux of organic anions from tubule cell cytosol to interstitial fluids. OAT2 transports many organic anions, including PAH, methotrexate, ochratoxin A and glutarate. Human, mouse, and rat orthologs of OAT2 have high affinities for the endogenous prostaglandin, PGE2 (Giacomini and Sugiyama, 2006).

- OAT3 (*SLC22A8*) was cloned originally from rat kidney. Human OAT3 consists of two variants, one of which transports a wide variety of organic anions, including PAH and estrone sulfate. The longer OAT3 in humans, a 568-amino-acid protein, does not support transport. It is likely that the two OAT3 variants are splice variants. Northern blotting suggests that the human ortholog of OAT3 is primarily in the kidney. Mouse and rat orthologs show some expression in the brain and liver. OAT3 mRNA levels are higher than those of OAT1, which in turn are

higher than those of OAT2 or OAT4. Human OAT3 is confined to the basolateral membrane of the proximal tubule (Giacomini and Sugiyama, 2006).

- OAT4 (*SLC22A9*) was cloned from a human kidney cDNA library. Quantitative PCR indicates that the expression level of OAT4 in human kidneys is approximately 5% to 10% of the level of OAT1 and OAT3 and is comparable with OAT2. OAT4 is expressed in human kidney and placenta; in human kidney, OAT4 is present on the luminal membrane of the proximal tubule. At first, OAT4 was thought to be involved in the second step of secretion of organic anions, i.e., transport across the apical membrane from cell to tubular lumen. However, recent studies demonstrate that organic anion transport by OAT4 can be stimulated by transgradients of α-ketoglutarate, suggesting that OAT4 may be involved in the reabsorption of organic anions from tubular lumen into cell. The specificity of OAT4 is narrow but includes estrone sulfate and PAH. Interestingly, the affinity for PAH is low (>1 mM). Collectively, emerging studies suggest that OAT4 may be involved not in secretory flux of organic anions but in reabsorption instead (Giacomini and Sugiyama, 2006).

- Other Anion Transporters. URAT1 (*SLC22A12*), first cloned from human kidney, is a kidney-specific transporter confined to the apical membrane of the proximal tubule, that URAT1 is primarily responsible for urate reabsorption, mediating electroneutral urate transport that can be transstimulated by Cl–gradients. The mouse ortholog of URAT1 is involved in the renal secretory flux of organic anions including benzylpenicillin and urate (Giacomini and Sugiyama, 2006).

2.5 Drug interactions

Drug interactions are a change in the way a drug acts in the body when taken with certain other drugs, herbals, or foods, or when taken with certain medical conditions. Drug interactions may cause the drug to be more or less effective, or cause effects on the body that are not expected (National Cancer Institute, USA). Since, the drug interactions have many reasons to classification, but a major contributor must be extent to which our knowledge of CYP isoforms has burgeoned, since altered drug metabolism is a major mechanism underlying many important drug interactions.

Types of drug interactions are frequently characterized as both pharmacokinetic and pharmacodynamic.

1) Pharmacokinetic drug interactions

Pharmacokinetic interactions are those in which one drug alters the rate or extent of absorption, distribution, metabolism or excretion of drug. This is most commonly measured by a change in one or more kinetic parameters, such as maximum concentration, area under the concentration-time curve, half-life, and total amount of drug excreted in urine, etc. The rate and extent of absorption can be affected by physiochemical factors such as complexation and non specific absorption of the drug and by physiologic factors such as gastrointestinal motility, gastrointestinal pH, gastrointestinal disease, gastric emptying time, intestinal blood flow, intestinal metabolism and induction or inhibition of transporters. However, most of the clinically important interactions involve formation of a non absorbable complex by either chelation or ion exchange.

Altered drug distribution in drug interactions is explained mainly by displacement of drug from plasma proteins or receptor binding sites. Protein binding displacement interactions after oral and intravenous dosing of low hepatically extracted drugs are generally clinically unimportant because unbound drug concentrations at steady state in plasma do not change or are transiently changed by displacement. Protein displacement interactions may cause adverse events if the displaced drug is highly bound (>95%) to plasma proteins at therapeutic concentrations and has a small volume of distribution and narrow therapeutic range.

Most clinically relevant interactions result from changes in drug elimination caused by inhibition or induction of metabolic enzymes present in the liver and extrahepatic tissues. These processes can cause changes in intrinsic clearance of elimination pathways, which result in alteration in unbound plasma concentrations at steady state for orally administration drugs. The concentrations of active metabolites can increase or decrease depending on weather their formation and elimination are directly or indirectly affected by inhibition or induction and thus influence weather a metabolite predisposes patients to drug toxicity or lack of drug effectiveness.

2) Pharmacodynamic drug interactions

Pharmacodynamic interactions are those in which one drug induces a change in a patient's response to a drug without altering the object drugs pharmacokinetics. This is one may see a change in drug action without altered plasma concentration. For example of this change is the increase in the toxicity of digoxin produced by potassium-wasting diuretics.

Pharmacological interactions, which are concurrent use of two or more drugs with similar or opposing pharmacological action are a form of pharmacodynamic interactions.

Drug-drug interactions

Drug-drug interaction is a modification of the effect of a drug when administered with another drug. The effect may be an increase or a decrease in the action of either substance, or it may be an adverse effect that is not normally associated with either drug.

Classifying of drug-drug interactions mechanistically gives major insight into how to predict, detect, and avoid them are follows (Aronson, 2004):

1) Pharmaceutical interactions
2) Pharmacokinetic interactions
3) Pharmacodynamic interactions

Drug-food interactions

Drug-food interactions are the effect produced when some drugs and certain foods or beverages are taken at the same time. For example, grapefruit juice blocks the metabolism of some drugs in the GI tract, an action that can cause normal dosages of a drug to reach toxic levels in the plasma. Drug-food interactions can be a major source of patient inconvenience and nonadherence through disruptions in a patient daily schedule. Unless advised to the contrary, patients often take drugs with meals as a suitable adherence reminder and to lesson gastrointestinal side effects (Harris, 2001).

Drug-herbal interactions

Herbs have been used for medicinal purposes since the beginning of recorded time. About one third of our drugs (including digitalis, morphine, atropine and several chemotherapeutic agents) were developed from plants. So, indeed, herbs can be potent products. Herbs can affect body functions; therefore, when herbs are taken concurrently with drugs, interactions are possible. The mechanism of action of many herbs has not been determined. Therefore, the exact mechanisms of drug-herb interaction are also unknown (Kuhn, 2002).

Drug-disease interactions

Drug-disease interactions are occurring when a disease alters the pharmacokinetics or pharmacodynamic of a drug. Such interactions can be classified mechanistically in an analogous fashion to drug-drug interactions. The study of Lindblad et al. (2005) and Doubova et al. (2007) were reported about 40.1% and 64% of patients had prescriptions implying one or more potential drug disease interactions in Frail, Hospitalized Elderly Veterans and in family medicine clinics in Mexico City, respectively. The most common potential interactions were calcium-channel blockers and heart failure and β-blockers and diabetes (Lindblad *et al*., 2005).

Drug-cytokine interactions

Drug-cytokine interactions has only recently been coined, the recognition of an interaction between the acute-phase response and hepatic drug metabolism. The acute-phase response to infection or injury, with is complex cascade of cytokines, endocrine hormones, free oxygen radicals, arachinodic acid metabolites,

catecholamines, reactive oxygen species and nitric oxide can have multiple effects on the pharmacokinetics and pharmacodynamic of many drugs (Haas, 2003).

2.5.1 Ciprofloxacin drug interactions

Ciprofloxacin is an inhibitor of CYP1A2 and 3A4 in human and rats microsomes (Mclellan *et al*. 1996). In addition, that is efficiently transported ABCG2 substrares (Alvarez *et al*., 2008). Other reported has been show that ciprofloxacin can accumulated in J774 macrophages; over expression and/or increased activity of the MRP-like ciprofloxacin transporter expressed at a basal level both the intracellular pharmacokinetics and activity (Michot *et al*., 2006).

Clozapine: In two cases reports, the first case receiving a daily dose of clozapine 900 mg and was admitted to hospital with urosepsis and was treated with a 5-day course of ciprofloxacin and amoxicillin. Two days after completion of antibacterial therapy, the patient developed symptoms of rhabdomyolysis. Clozapine therapy was discontinued and measurement of the patient's clozapine plasma concentration 1 day after cessation of clozapine therapy and 3 days after cessation of ciprofloxacin treatment showed that it was in excess of recommended therapeutic levels. The second case was treated with a daily dose of clozapine 300 mg and was admitted to hospital because of delirium and suspected urinary tract infection or pneumonia. Treatment with ciprofloxacin was initiated. Measurement of clozapine plasma concentrations prior 2 and 3 days after commencement of ciprofloxacin showed that clozapine concentrations doubled over that time period. This result show that inhibition of cytochrome P450 (CYP) enzymes 1A2 and 3A4 by ciprofloxacin resulted in delayed clozapine metabolism and elevated clozapine plasma concentrations (Brouwers *et al*., 2009).

Cyclosporine: Borras-Blasco et al. (2005) studied the effects of cyclosporine in a 38-year-old man diagnosed with pure red blood cell aplasia was undergoing treatment with cyclosporine 200 mg/day and up to 250 mg/day on day 41. On day 45, the patient was hospitalized with fever, and ciprofloxacin 200 mg IV three times a day was begun. The level of cyclosporine was increase 297 ng/mL after ciprofloxacin was added and the cyclosporine blood levels was decrease after the withdrawal of ciprofloxacin suggest a potential interaction.

Methadone: in patient who treated with oral methadone 140 mg/day for more than 6 years and received oral ciprofloxacin 750 mg twice daily for two days because of urocepsis. The patients become sedation, confusion and respiratory depression an extended hospital stay. These side effects were caused by ciprofloxacin inhibition the metabolism of methadone via cytochrome P450 isozymes (Herrlin *et al*., 2000).

Sildenafil: Hedaya et al. (2006) studied in healthy volunteers, showed that the AUC and C_{max} of a single 50 mg dose of sildenafil were significantly increased by 110% and 117%, respectively when received one tablet of ciprofloxacin (500 mg).

Ketoconazole or Itraconazole: Abou-Auda et al. (2008) studied the effects of the concomitant administration of the antifungal drugs ketoconazole (KTC) or itraconazole (ITC) on the pharmacokinetics of ciprofloxacin (CIP) following short and long-term administration in mice was investigated. The concomitant administration of KTC or ITC with CIP also significantly (p<0.05) increased C_{max}, $T_{1/2}$, mean residence time (MRT) and $AUC_{(0-\infty)}$ with no change in T_{max}. CIP clearance was significantly reduced by both agents.

2.5.2 Itraconazole drug interactions

Itraconazole is mainly metabolised through CYP3A4. Its also a potent inhibitor of CYP3A4 and P-gp (Venkatakrishnan *et al*., 2000). The liver is the primary site of drug metabolism mediated by the cytochrome P-450 system, but CYP3A4 is also present in the enterocytes of the small intestine, placenta, kidney, brain and liver. The enterocytes in the intestinal mucosa are also a major site of expression of P-gp. P-gp is an ATP-dependent plasma membrane transporter that is an important molecular determinant of oral bioavailability and brain penetration.

Fexofenadine: Shimizu et al. (2006) studied the effects of single and multiple doses of itraconazole on the pharmacokinetics of fexofenadine, a substrate of P-gp. Itraconazole was administered orally for 6 days and on days 1, 3 and 6, fexofenadine was administered simultaneously. On another occasion, fexofenadine was administered alone. They found that itraconazole increased fexofenadine

$AUC_{(0,\infty)}$ and the percent change for difference was 178%, 205% and 169% on days 1, 3 and 6 of the 6 day treatment, respectively.

Budesonide: In a randomized, double-blind, 2-phase crossover studied in healthy subjects, treated with 200 mg of itraconazole and received 1000 µg of inhaled budesonide after the last dose of itraconazole. They found that itraconazole markedly increased budesonide concentrations, the mean AUC, peak plasma concentrations of inhaled budesonide 4.2-fold and 1.6-fold (p<0.01) respectively, compared with placebo. Moreover, the mean terminal half-life of inhaled budesonide was prolonged from 1.6 to 6.2 hours (p<0.001) by itraconazole. The suppression of cortisol production after inhalation of budesonide was significantly increased when compared with placebo, about 43% reduction in the area under the plasma cortisol concentration–time curve from 0.5 to 10 hours (p<0.001) and a 12% decrease in the cortisol concentration measured 23 h after administration of budesonide (Raaska *et al*., 2002).

Loperamide: Niemi et al. (2006) studied the effects of itraconazole, an inhibitor of CYP3A4 and gemfibrozil, an inhibitor of CYP2C8 on the pharmacokinetics of loperamide, which is metabolized in *in vitro* via CYP2C8, CYP3A4 and also substrate of P-gp efflux transporter in healthy volunteers. All subjects received 100 mg itraconazole (first dose 200 mg), 600 mg gemfibrozil twice daily for 5 days. On day 3, each subjects received a single 4 mg of loperamide. They found that itraconazole, gemfibrozil and their combination markedly raise the plasma concentrations of loperamide.

Quinidine: In a treatment dose trial in healthy volunteers to assess the pharmacokinetics of quinidine (substrates of CYP3A4 and P-gp) given before and during treatment with itraconazole showed that it increased an average the peak plasma concentration of quinidine to 1.6-fold (p<0.05) and increased the AUC of quinidine to 2.4-fold (p<0.01) by itraconazole. The elimination half-life of quinidine was prolonged 1.6-fold (p<0.001) and the area under the 3-hydroxyquinidine/quinidine ratio-time curve decreased to one-fifth (p<0.001) and the renal clearance was decreased about 50% (p<0.001) by itraconazole (Kaukonen *et al*., 1997).

Dexamethasone: In a treatment phase in healthy subjects. Itraconazole markedly increases the plasma concentrations of dexamethasone (substrate of CYP3A4) and enhances its adrenal-suppressant effect showed that itraconazole decreased the systemic clearance of IV administered dexamethasone by 68% (p<0.001), increased the $AUC_{(0-\infty)}$ 3.3-fold (p<0.001) and prolonged the elimination half-life about 3.2-fold (p<0.001). The $AUC_{(0-\infty)}$ of oral dexamethasone was increased 3.7-fold (p<0.001) the peak plasma concentration 1.7-fold (p<0.001) and the elimination half-life 2.8-fold (p< 0.001) by itraconazole (Varis *et al.*, 2000).

Morphine: Heiskanen et al. (2008) studied the efflux transporter; P-gp can affect gastrointestinal absorption and tissue distribution of oral morphine in 12 healthy volunteers, randomized crossover study, once daily 200 mg itraconazole or placebo for 4 days. On day 4, 1 h after the last pretreatment dose, the subjects ingested 0.3 mg/kg morphine. They found that itraconazole moderately increases the plasma concentrations of oral morphine. The $AUC_{(0–9)}$, $AUC_{(0–48)}$ of morphine was increased by 29% and 22% respectively, probably by enhancing its absorption by inhibiting intestinal wall P-gp.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Drugs

Ciprofloxacin tablets (Ciprobay®, Lot No. 2405A1D) were purchased from Bayer (Thailand) Limited, under license of Bayer HealthCare AG, Germany.

Itraconazole capsules (Sporal®, Lot No. 843002) were purchased from Olic (Thailand) Limited, under contract with Janssen-Cilag Ltd.

3.1.2 Chemicals and reagents

Standard ciprofloxacin hydrochloride (Lot No. 030832) was purchased from Zhejing Jingxin Pharmaceutical Company, People Republic of China.

Quinine sulfate was kindly donated by the Nutritional Biochemicals Corp, USA, which was the internal standard of ciprofloxacin.

Standard itraconazole (Lot No. Stan-9604-007-1) and internal standard (R51012) (Lot No. Stan-9404-029-1) were purchased from Fitzgerald Industrials International Institute, USA.

Methanol and acetonitrile (HPLC grade) were purchased from J.T. Baker NJ, USA.

Potassium dihydrogen phosphate (KH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Merck Darmstadt, Germany. Water was purified by Milli Q Water Purification System, Millipore, Milford, MA, USA.

3.2 Equipments and columns

3.2.1 HPLC model

a) Waters 2695 pump, autosampler (Waters Associates, Milford, USA)
b) Waters 2475 Multi λ Fluorescense Detector (Waters Associates, Milford, USA)
c) Waters 2487 Dual λ Absorbance Detector (Waters Associates, Milford, USA)
d) Empower software Version 5 (Waters Associates, Milford, USA)
e) μ-Bondapack™ C_{18} column: reverse-phase column C_{18}, 3.9 mm x 300 mm, particle size 10 μm. (Waters Associates, Milford, MA, USA)
f) Symmetry® C_{18} column: reverse-phase column C_{18}, 3.9 mm x 150 mm, particle size 5 μm, (Waters Associates, Milford, MA, USA)
g) μ-Bondapack™ C_{18} guard column: packed with resolved C_{18} (Waters Associates, Milford, MA, USA)

3.2.2 Instruments

a) Vortex mixer
b) Centrifuge machine
c) pH meter
d) Micropipette (100, 200, and 1,000 μL)
e) Pipette tip
f) Disposable needle (21-22G)
g) Heparin lock
h) Test tube with cap
i) Disposable syringe (3 mL, 5 mL, and 10 mL)
j) Eppendorf microcentrifuge tube (1.5 mL)
k) PTFE filter, pore size 0.45 μm

3.3 Methodology

3.3.1 Determination of ciprofloxacin concentrations in plasma

Sample preparation

Fifty microliters of 10,000 ng/mL of internal standard (quinine sulfate dissolved in 20% methanol) was added to 500 µL of plasma sample and was deproteinized with 500 µLof acetonitrile. The mixture was vortexed for 30 sec and centrifuged at 14,000 rpm for 15 min. Two hundred microliters of supernatant was transferred to 800 µL of mobile phase and then the mixture was vortexed for 30 sec. Twenty microliters of the supernatant was injected onto the HPLC system for analysis.

Chromatographic condition

Plasma ciprofloxacin concentrations were determined by HPLC. The assay method was modified from Jim et al. (1992) using the following conditions:

Column	:	µ-Bondapack™ C_{18} column: reverse-phase column C_{18}, 3.9 mm x 300 mm, particle size 10 µm
Guard column	:	µ-Bondapack C_{18} pack with resolved C_{18}
Mobile Phase	:	Mixture of methanol: phosphate buffer (0.08 mM KH_2PO_4: 0.06 mM Na_2HPO_4) was adjusted to pH 3 with 85% H_3PO_4 and tetrahydrofuran
Flow rate	:	1.2 mL/min
Injection volume	:	20 µL
Detector	:	Fluorescence, excitation and emission wavelengths of 277 and 440 nm
Temperature	:	50 ºC

Mobile phase

The mobile phase which consisted of methanol and phosphate buffer (0.08 mM KH_2PO_4:0.06 mM Na_2HPO_4) was adjusted to pH 3 with 85% H_3PO_4 and tetrahydrofuran (21.2: 39.0: 39.0: 0.8 v/v/v). The mobile phase was freshly prepared daily and filtered through PTEF filter paper, pore size 0.45 μm, and degassed in the ultrasonic bath for 30 min before using.

Standard curves

The working standard solution (16,000 ng/mL) was diluted to serial concentrations of 31.25, 125, 1,000, 4,000 and 8,000 ng/mL with drug-free plasma. Standard calibration curves were constructed by the least-square linear regression of ciprofloxacin concentration and peak area ratio of ciprofloxacin. Unknown concentrations of ciprofloxacin in volunteer's plasma were calculated from the standard curves by reverse prediction.

Stock standard and internal standard solutions

The stock standard ciprofloxacin and internal standard quinine sulfate solutions at a concentration of 1,000 μg/mL were prepared in methanol and stored at -70ºC under light protecting condition for no more than 4 months before use. The working solution was prepared in deionized water. The stock solutions of ciprofloxacin stored at 5ºC under light protecting condition was stable for 1 month, whereas diluted standard water solution was only stable for 6 h at room temperature and daylight (Vybiralova *et al*., 2005). The extracted samples of ciprofloxacin were stable at 20ºC for 15 h prior to analysis (Smet *et al*., 2009). Working standard solutions of ciprofloxacin used to prepare a standard calibration curve was freshly prepared daily by diluting the stock solution with blank plasma.

3.3.2 Determination of itraconazole concentrations in plasma

Sample preparation

Five hundred microliters of plasma was added with 25 µL of 10,000 ng/mL internal standard (R51012, dissolved in 20% methanol) and deproteinized with 500 µl of acetonitrile. The mixture was vortexed for 30 sec and centrifuged at 14,000 rpm for 15 min. Two hundred microliters of supernatant was injected onto the HPLC system for analysis.

Chromatographic condition

Plasma itraconazole concentrations were determined by HPLC. The assay was modified from Badcock (1990) using the following conditions:

Column	:	Symmetry® C_{18} column: reverse-phase column C_{18}, 3.9 mm x 150 mm, particle size 5 µm
Guard column	:	µ-Bondapack™ C_{18} pack with resolved C_{18}
Mobile Phase	:	Mixture of acetonitrile and water (60:40 v/v) was added with 300 µL/L of diethylamine and pH was adjusted to 7.8 with 85% H_3PO_4
Flow rate	:	1.0 mL/min
Injection volume	:	200 µL
Detector	:	Absorbance detector, wavelengths of 263 nm, Gain 10 and AUFS 0.002
Temperature	:	Ambient (25 ± 1ºC)

Mobile phase

The mobile phase consisted of acetonitrile and water (60: 40 v/v) was added with 300 µl per liter of diethylamine and pH was adjusted to 7.8 with 85% H_3PO_4. The mobile phase was freshly prepared and filtered through PTEF filter paper, pore size 0.45 µm, and degassed in the ultrasonic bath for 30 minutes before using.

Standard curves

The working standard solution (12,800 ng/mL) was diluted to serial concentrations of 50, 100, 200, 400, 800, 1,600, 3,200 and 6,400 ng/mL with drug-free plasma. Standard calibration curves were constructed by the least-square linear regression of itraconazole concentration and peak area ratio of itraconazole. Unknown concentrations of itraconazole in volunteer's plasma were calculated from the standard curves by reverse prediction.

Stock standard and internal standard solution

The stock standard itraconazole and internal standard (R51012) solutions at a concentration of 1,000 μg/mL were prepared in methanol. The working solution prepared in 20% methanol and stored at -70°C under light protecting condition was stable for no more than 6 months. Methanolic solutions of itraconazole have been reported to be stable at 4°C for at least 12 months and serum samples were stable for more than 6 months at -20°C (Compas *et al*., 1996; Srivatsan *et al*., 2004). Working standard solutions used to prepare a standard calibration curve were freshly prepared by diluting the solution with drug-free plasma.

3.3.3 Method validation

The method validation was done according to the guidance of the US FDA, 2001 and Thai FDA, 2006.

Recovery study

Recovery of plasma ciprofloxacin and itraconazole was determined by comparing peak area of deproteinized drug in plasma with peak area of the drug in mobile phase. An acceptable recovery should be more than 90% and coefficient of variation (CV) should be less than 5%.

$$\text{Recovery (\%)} = \frac{\text{Peak area ratio of standard ciprofloxacin /itraconazole in plasma}}{\text{Peak area ratio of standard ciprofloxacin /itraconazole in mobile phase}} \times 100$$

Precision and accuracy

To determine intraday precision and accuracy, drug-free plasma was spiked with ciprofloxacin standard solution at 250, 2,000 and 6,000 ng/mL and with itraconazole standard solution at 50, 100, 800 and 3,200 ng/mL. Five replications of each were carried out in one day.

To determine interday precision and accuracy, drug-free plasma was spiked with ciprofloxacin standard solution at 250, 2,000 and 6,000 ng/mL and with itraconazole standard solution at 50, 100, 800 and 3,200 ng/mL. Two replications of each were carried out in five days. The % CV and accuracy of intraday and interday determination for each concentration should be less than 15%.

$$\% \text{ CV} = \frac{\text{Standard deviation (SD)}}{\text{Mean value}} \times 100$$

$$\text{Accuracy (\%)} = \frac{\text{Calculated concentration}}{\text{Actual concentration}} \times 100$$

Lower limit of quantification

Lower limit of quantification was obtained by adding a known amount of ciprofloxacin or itraconazole to drug-free plasma (31.25-8,000 ng/mL and 50-6,400 ng/mL, respectively) and deproteinized as described above. The concentration of ciprofloxacin or itraconazole and peak area ratio was plotted for correlation. The lowest concentration of ciprofloxacin and itraconazole which was still linearly correlated was regarded as low limit of quantification (LLOQ).

3.3.4 Sample size calculation

The study was conducted to determine the influence the pharmacokinetic interaction of ciprofloxacin and itraconazole in normal volunteers. However, there were no reports on the interaction of ciprofloxacin and itraconazole in humans. The study of Abou-Auda et al. (2008) on the pharmacokinetic interaction of ketoconazole and itraconazole with ciprofloxacin in mice was therefore used to

calculate the sample size. They found that the C_{max} of ciprofloxacin is significantly reduced from 3.01 to 2.20 μg/.mL^{-1} when co-administered with itraconazole (SD = 0.42 μg/mL).

The C_{max} difference of ciprofloxacin (d) = 3.01-2.20 = 0.81 μg/mL. Type I error 5% ($\alpha = 0.05$), $Z_\alpha = 1.645$, and Type II error 10% ($\beta = 0.10$), $Z_\beta = 1.282$

$$N = \frac{2(Z_\alpha + Z_\beta)^2 (SD)^2}{d^2}$$

$$= \frac{2(1.645 + 1.282)^2 (0.42)^2}{(0.81)^2}$$

$$= 4.61$$

$$\approx 5$$

A total sample size of 5 volunteers should be sufficient to detect a significant pharmacokinetic difference in C_{max} for ciprofloxacin. However, this sample size was too small and would create potential for an error in statistical analysis. Moreover, it is necessary to increase the sample size to offset the dropouts and withdrawals from the study. Therefore, the sample size of volunteers used in this study was adjusted upward to ten.

3.3.5 Pharmacokinetic study

Volunteers

All volunteers were given the detailed explanation of the purpose, protocol and risk of the study, and a written consent was obtained from each volunteer prior to the study. The protocol was approved by the Ethics Committee of the Faculty of Sciences, Prince of Songkla University, Hat-Yai, Thailand (Appendix C). Ten Thai male healthy volunteers, age 19-32 y old (mean age ± SD, 24.7 ± 4.29 y), weighed 50-80 kg (mean weight ± SD, 61.6 ± 11.5 kg) with body mass index (BMI) of 18.29 to 24.42 kg/m^2 was (mean BMI ± SD, 21.48 ± 2.54 kg/m^2) participated in the study. Prior to the study, medical history, physical examination, standard biochemical and hematological screening test (complete blood count, BUN, serum creatinine, SGOT, SGPT, total bilirubin, alkaline phosphatase, fasting blood glucose, total protein and

albumin) were examined in each volunteer. The volunteers were not allowed to take any medications, smoke cigarettes, or ingest alcohol and caffeine-containing beverages for one month before starting the study and during the study period.

Study design

The study was an open-labeled, randomized, two-phase crossover design with a 2-week washout period. The ten healthy subjects were divided into 2 groups. In the first phase, all subjects received 500 mg (1 tablet) of ciprofloxacin or 200 mg (2 capsules) of itraconazole. In the second phase, all subjects received 200 mg itraconazole (2 capsules) with 500 mg ciprofloxacin (1 tablet).

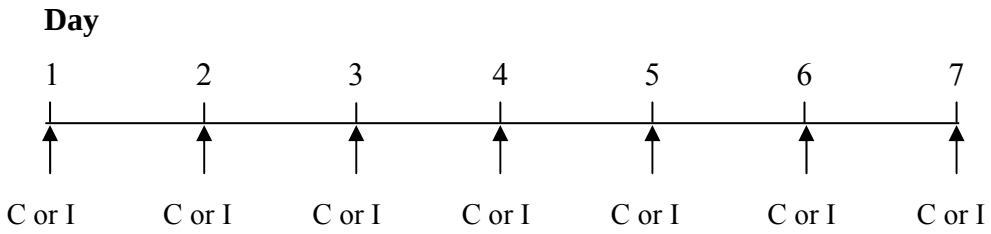
Phase 1. Each subject received 200 mg itraconazole (2 capsules) or 500 mg ciprofloxacin (1 tablet) orally after breakfast (8 am) and dinner (8 pm) with a glass of water (200 mL) for 7 days. No milk was allowed as substitution of water while taking the drugs. In the morning of day 7 after an overnight fast, each subject received an oral dose of 200 mg itaconazole (2 capsules) or 500 mg ciprofloxacin (1 tablet) with 200 mL of water after a standardized breakfast. No food was allowed for at least 4 h after drug administration.

A catheter was inserted into a forearm vein for collection of blood samples and was maintained patent using 1 ml of heparin solution (100 IU) after each blood sample collection. Venous blood samples (5 mL) were collected into heparinized (20 μL per 5 mL) tubes before drug administration and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 24, and 48 h for ciprofloxacin and up to 96 h for itraconazole after drug administration. Samples were centrifuged at 3,000 rpm for 15 min and the plasma was collected and stored at -70 ºC until analysis.

Phase 2. After two-week washout period, all subjects orally received 200 mg itaconazole (2 capsules) with 500 mg ciprofloxacin (1 tablet) twice daily for 7 days. In the morning of day 7 after an overnight fast, each subject received 200 mg itaconazole (2 capsules) with 500 mg ciprofloxacin (1 tablet) orally with 200 mL of water after a standardized breakfast. No food was allowed for at least 4 h after drug administration. The blood samples were collected in the same manner as previously described in phase 1

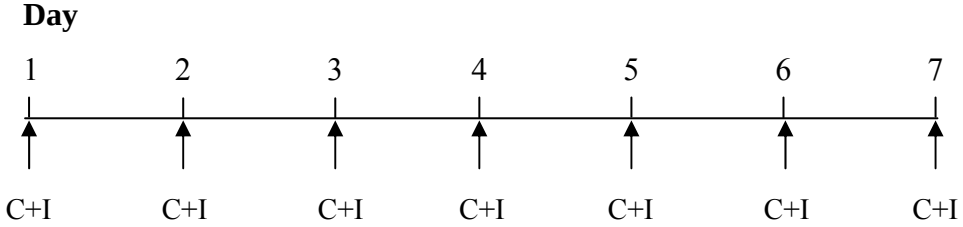
Schematic plan of study

Phase 1: A multiple oral dose of ciprofloxacin or intraconazole



Blood collection for 48 h for ciprofloxacin and up to 96 h for itraconazole

Phase 2: A multiple oral dose of ciprofloxacin with itraconazole



Blood collection for 96 h (0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 24, 48, 72 and 96 h)

Remarks: **C = Ciprofloxacin (500 mg, orally twice daily for 7 days)**

I = Itraconazole (200 mg, orally twice daily for 7 days)

Pharmacokinetic analysis

Pharmacokinetic parameters (AUC_{0-t}, $AUC_{0-\infty}$, $T_{1/2}$, C_{max}, T_{max}, CL/f, MRT and λ_z) were analyzed by non-compartment model with the use of WinNonlin Professional Software Version 1.1 (Pharsight, Mountain View, CA).

Statistical analysis

All pharmacokinetics parameters were expressed as mean ± SD. The data showed normal distribution, therefore, parametric statistic test was used for data assessment. Paired *t*-test was applied for pairwise comparisons. Significant level was set at *p*-value of less than 0.05.

CHAPTER 4

RESULTS

4.1 Chromatograms

The chromatograms showed that the peaks of ciprofloxacin and its internal standard quinine sulfate and itraconazole and its internal standard (R51012) were well separated from the other peaks in mobile phase and in plasma as shown in Figures 13 and 14. The standard ciprofloxacin and internal standard quinine sulfate were eluted at around 9 and 11 min, respectively. The standard itraconazole and internal standard (R51012) were eluted at around 8 and 11 min, respectively. Representative chromatograms of ciprofloxacin and itraconazole in plasma are illustrated in Figures 15 and 16, respectively.

4.2 Assay validation

4.2.1 Linearity of the standard calibration curve

The correlation coefficient (r^2) of the standard curves of ciprofloxacin and itraconazole were 0.9999 and 0.9993, respectively. The linear regression equation of ciprofloxacin and itraconazole were Y = 0.0072X - 0.2419 and Y = 0.01X - 0.3676, respectively (Figures 17 and 18).

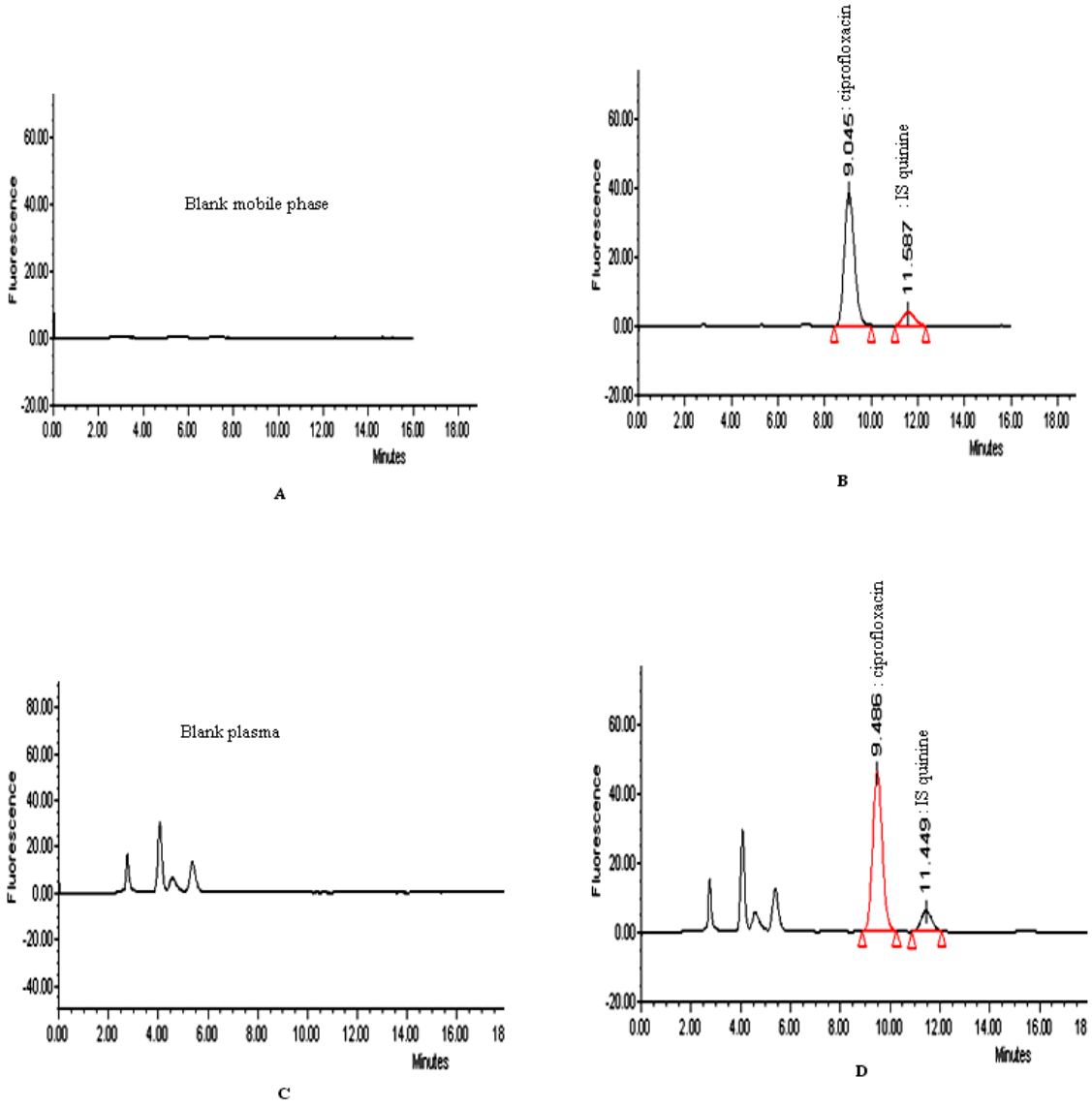


Figure 13. Chromatograms of standard ciprofloxacin and internal standard quinine sulfate in 500 µl mobile phase and plasma. (A) blank mobile phase; (B) spiked with standard ciprofloxacin (1000 ng/mL, retention time 9.045 min) and internal standard quinine sulfate (10000 ng/mL, retention time 11.587 min); (C) blank plasma; (D) spiked with standard ciprofloxacin (1000 ng/mL, retention time 9.486 min) and internal standard quinine sulfate (10000 ng/mL, retention time 11.449 min). Flow rate was 1.2 mL/min.

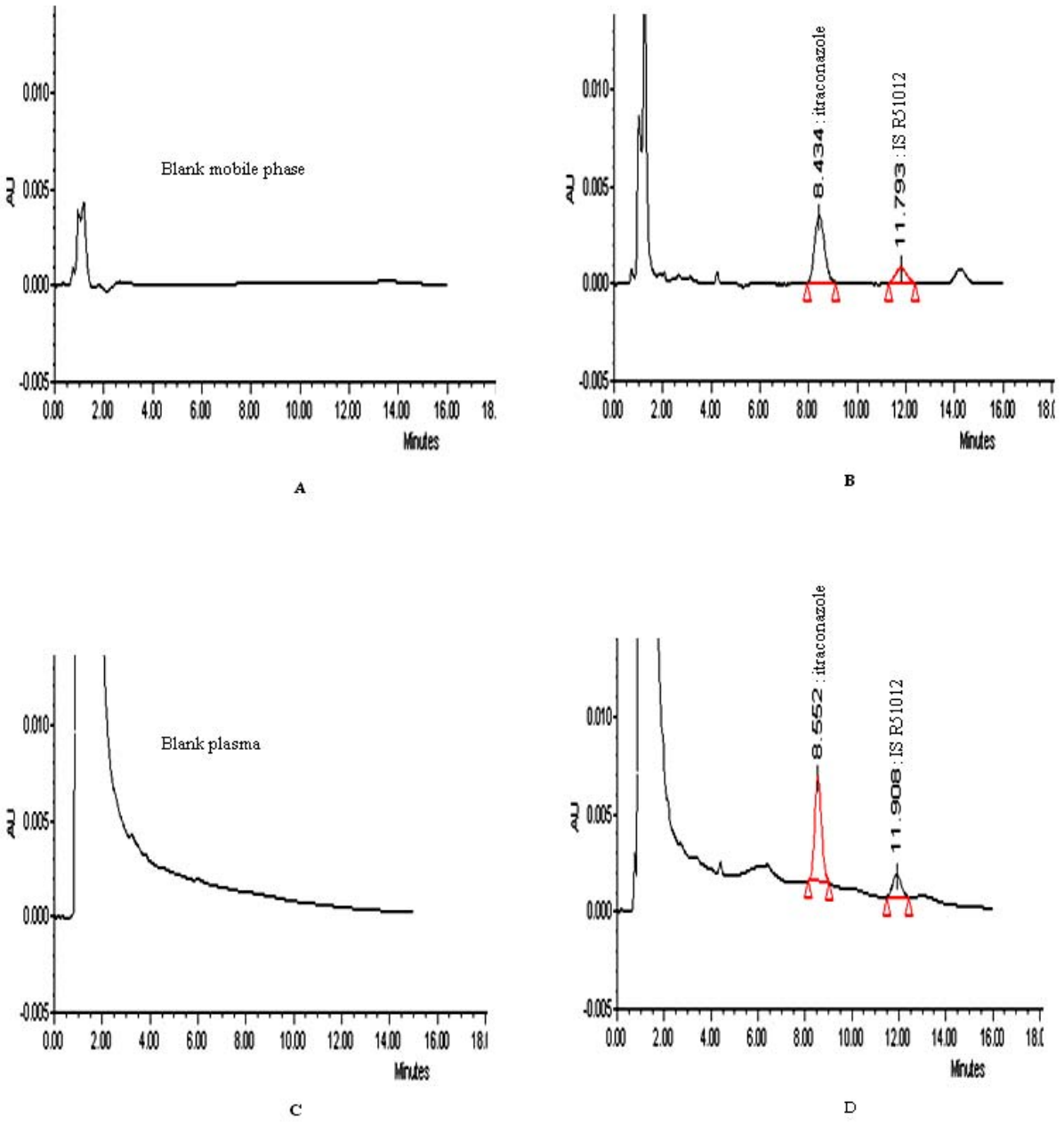


Figure 14. Chromatograms of standard itraconazole and internal standard (R51012) in 500 µl mobile phase and plasma. (A) blank mobile phase; (B) spiked with standard itraconazole (800 ng/mL, retention time 8.434 min) and internal standard R51012 (10000 ng/mL; retention time 11.793 min); (C) blank plasma, (D) spiked with standard itraconazole (800 ng/mL, retention time 8.552 min) and internal standard R51012 (10000 ng/mL, retention time 11.908 min). Flow rate was 1 mL/min.

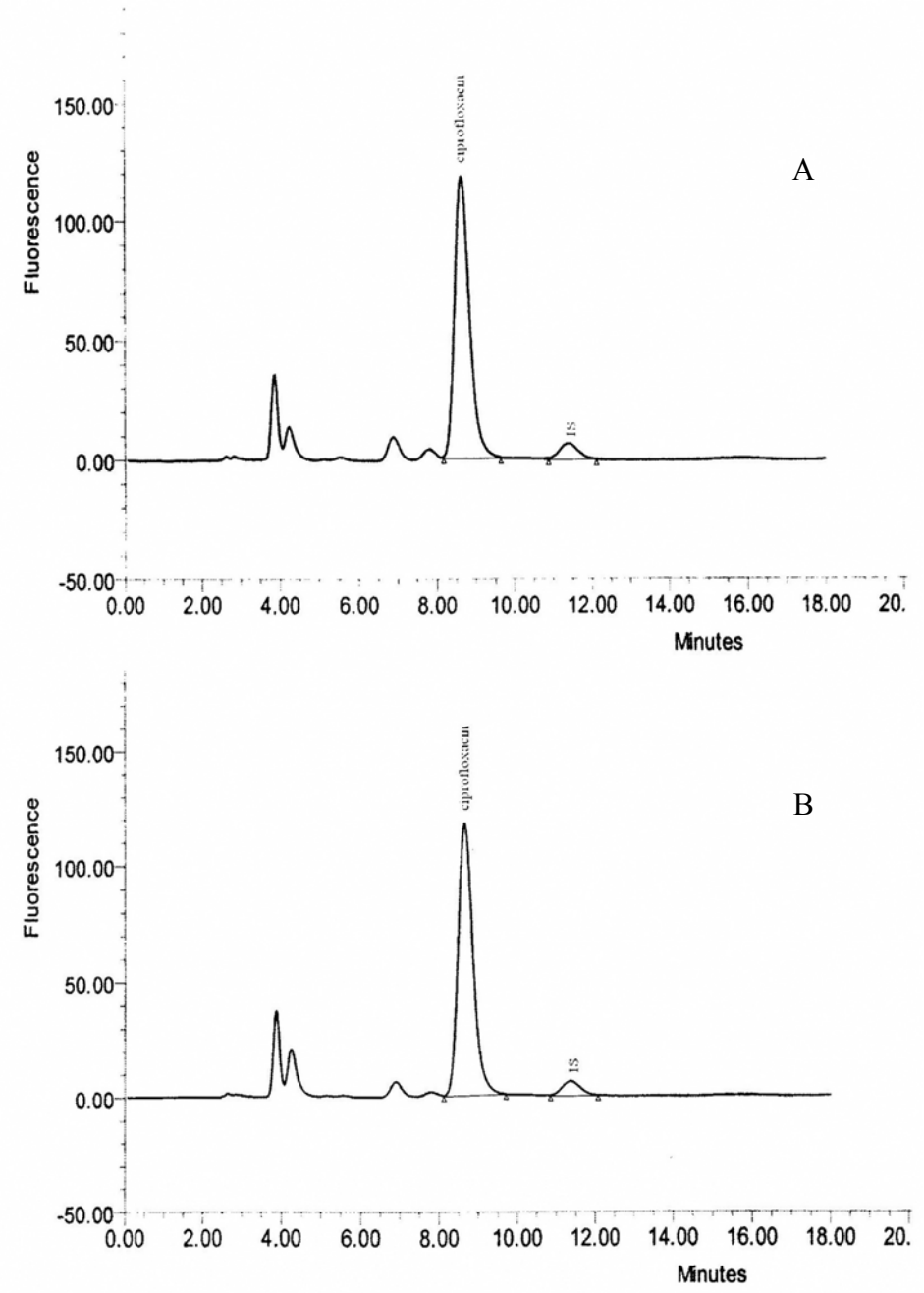


Figure 15. Representative chromatograms of ciprofloxacin in 50 μL human plasma. (A) plasma obtained from a subject receiving 500 mg ciprofloxacin at 0.5 h after drug administration; (B) plasma obtained from a subject receiving 500 mg ciprofloxacin with 200 mg itraconazole at 0.5 h after drug administration.

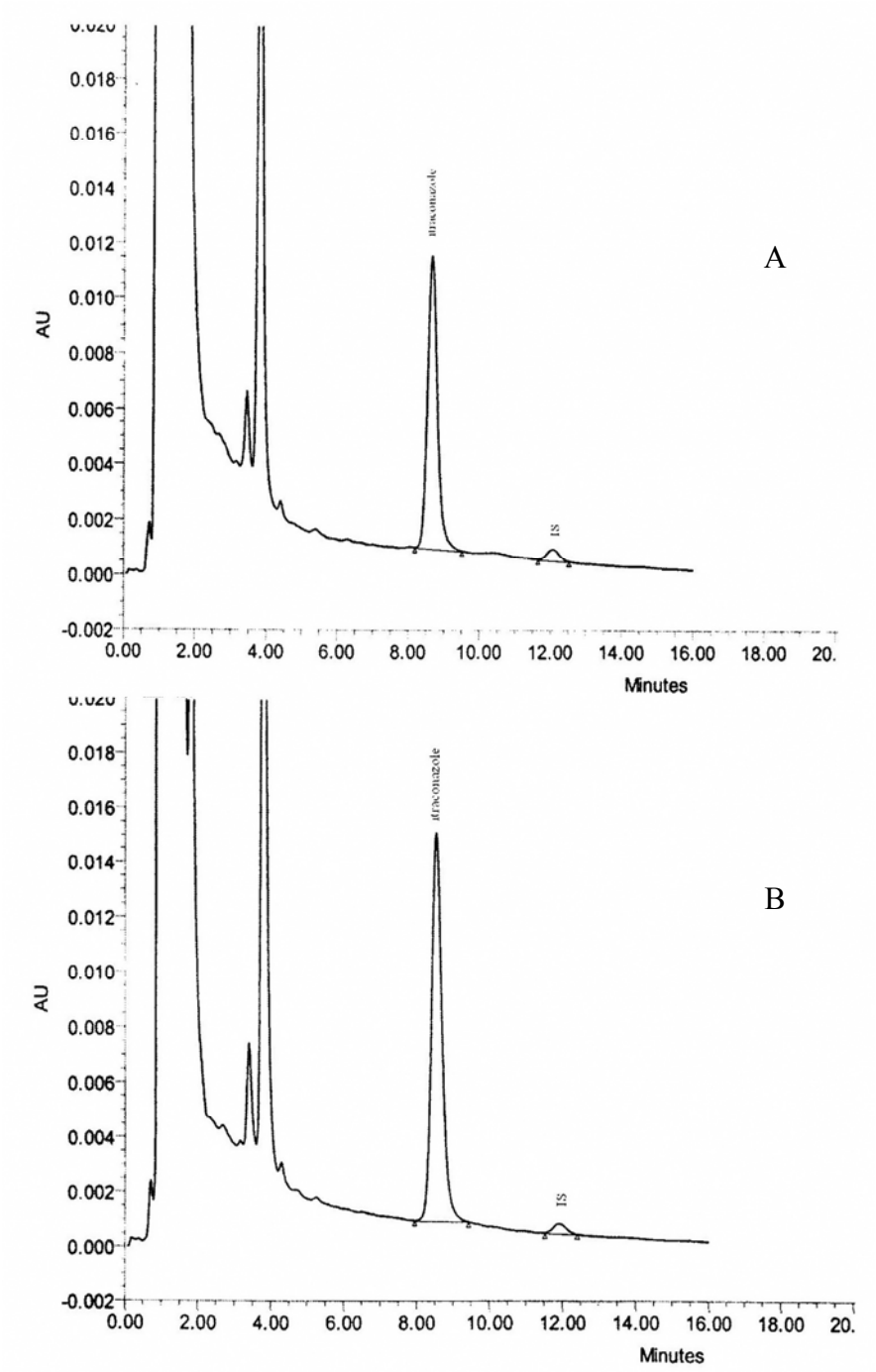


Figure 16. Representative chromatograms of itraconazole in 200 μL human plasma. (A) plasma obtained from a subject receiving 200 mg itraconazole at 0.25 h after drug administration; (B) plasma obtained from a subject receiving 200 mg itraconazole with 500 mg ciprofloxacin at 0.25 h after drug administration.

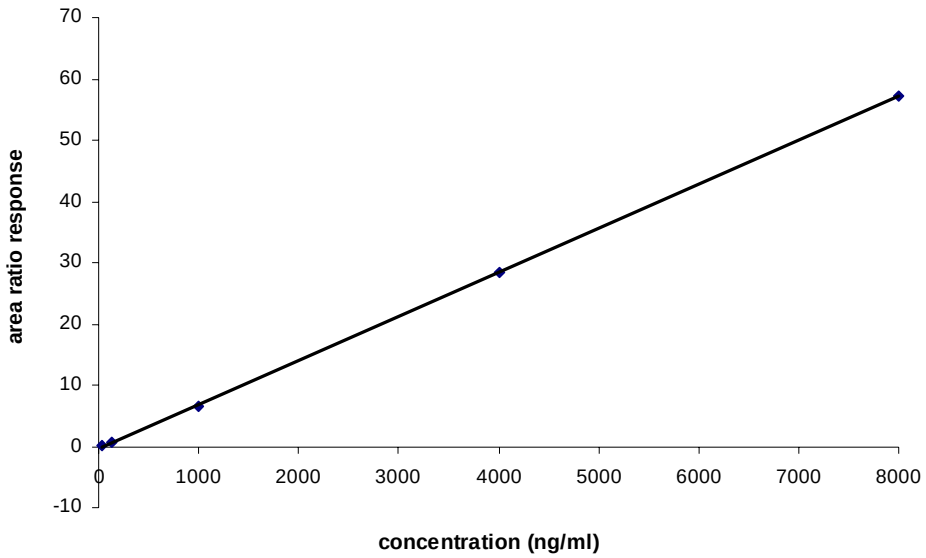


Figure 17. Calibration curve of ciprofloxacin in plasma (Y = 0.0072X - 0.2419) with $r^2 = 0.9999$.

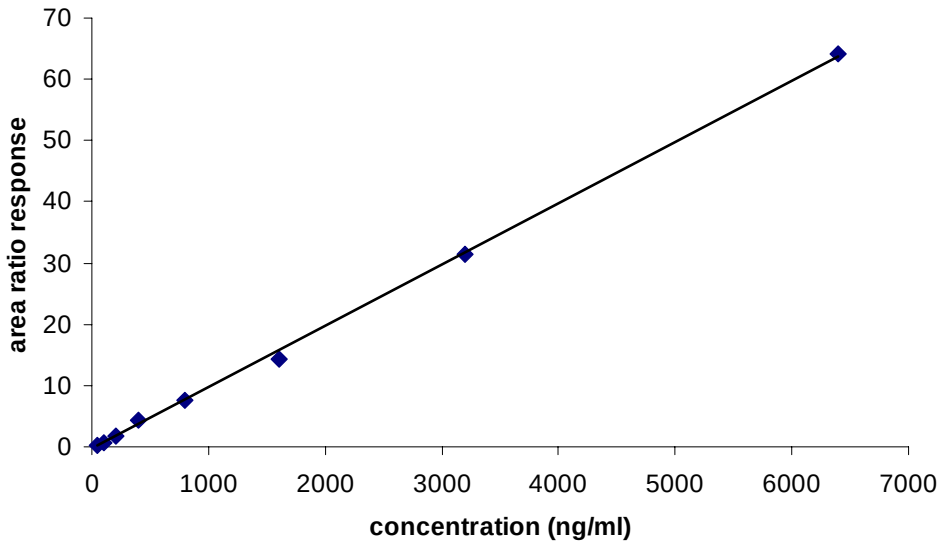


Figure 18. Calibration curve of itraconazole in plasma (Y = 0.01X - 0.3676) with $r^2 = 0.9993$.

4.2.2 Precision

Precision of the assay procedure was assessed from % CV of peak area ratio of ciprofloxacin or itraconazole and their concentrations from intraday and inter -day assay are shown in Tables 1 and 2. The % CV of intraday and interday precision of ciprofloxacin was 0.82-4.46% and 0.33-1.00%, respectively. The % CV of intraday and interday precision of itraconazole was 1.66-4.14% and 0.37-1.84%, respectively.

4.2.3 Recovery

Efficacy of deprotenization procedure was assessed from the percentage recovery as shown in Table 3. The mean recoveries of ciprofloxacin at concentrations of 250, 2000, and 6000 ng/mL were 84.54%, 85.35% and 96.35%, respectively. The mean recoveries of itraconazole at concentrations of 50, 800 and 3200 ng/mL were 66.70%, 88.51%, and 117.43%, respectively.

4.2.4 Accuracy

Accuracy of intraday and interday ciprofloxacin and itraconazole in plasma was controlled by determining three ciprofloxacin concentrations (250, 2000 and 6000 ng/mL) and three itraconazole concentrations (50, 800 and 3200 ng/mL) in five replicates for intraday and ten replicates for interday. The intraday accuracy of ciprofloxacin and itraconazole ranged between 96.01-108.61% and 95.57-105.07%, respectively. The interday accuracy of ciprofloxacin and itraconazole ranged between 94.73-104.44% and 93.15-100.45%, respectively (Tables 1 and 2).

4.2.5 Lower limit of quantification (LLOQ)

The LLOQ of ciprofloxacin and itraconazole were found to be 31.25 ng/mL and 50 ng/mL, respectively.

Table 1. The intraday and interday precision of the analytical method of ciprofloxacin

Concentration (ng/mL)	Mean concentration ± SD (ng/mL)	CV (%)	Accuracy (%)
Intraday (n=5)			
250	271.51 ± 2.21	0.82	108.61
2000	1920.14 ± 33.74	1.76	96.01
6000	6034.60 ± 269.27	4.46	100.58
Interday (n=10)			
250	261.09 ± 0.90	0.33	104.44
2000	1894.59 ± 18.83	1.00	94.73
6000	6012.63 ± 39.78	0.67	100.21

Table 2. The intraday and interday precision of the analytical method of itraconazole

Concentration (ng/mL)	Mean concentration ± SD (ng/mL)	CV (%)	Accuracy (%)
Intraday (n=5)			
50	52.54 ± 1.86	3.54	105.07
800	764.57 ± 31.69	4.14	95.57
3200	3240.43 ± 53.69	1.66	101.26
Interday (n=10)			
50	50.22 ± 0.40	0.80	100.45
800	745.17 ± 13.72	1.84	93.15
3200	3119.63 ± 11.56	0.37	97.49

Table 3. Recovery of ciprofloxacin and itraconazole in plasma

Drugs	Concentration (ng/mL)	Mean peak area ratio in plasma ± SD (n = 5)	Mean peak area ratio in mobile phase ± SD (n = 5)	% Mean recovery
Ciprofloxacin	250	1.56 ± 0.02	1.85 ± 0.07	84.54
	2000	13.91 ± 0.33	16.29 ± 0.21	85.35
	6000	46.40 ± 1.39	48.16 ± 1.23	96.35
Itraconazole	50	0.22 ± 0.005	0.32 ± 0.022	66.70
	800	3.99 ± 0.556	4.51 ± 1.354	88.51
	3200	18.63 ± 0.34	15.86 ± 0.31	117.43

4.3 Volunteers

All ten male healthy volunteers completed this study. Demographic and laboratory data of the volunteers are summarized in Table 4. No serious adverse effects were observed after drug administration. Common but mild adverse reactions were nausea (60%), dry mouth (40%), diarrhea (10%), and headache (10%).

Table 4. Demographic and laboratory data of ten healthy male volunteers in the study

Volunteer No.	Age (y)	BMI (kg/m^2)	WBC $Cell/mm^3$	Hb	Hct	Lymph %	Total bilirubin mg%	BUN mg%	Cr mg%	SGOT U/L	SGPT U/L	ALP U/L
1	30	24.34	6,500	14.6	43	42	1.0	11.0	1.2	17	22	82
2	21	19.72	6,700	14	43	54	0.8	6.3	1.1	15	17	109
3	25	24.42	7,000	15	45	49	0.9	7.7	1.3	14	10	104
4	27	19.29	5,200	14.3	44	43	1.0	13.7	1.0	11	11	45
5	25	18.29	6,700	14.4	44	33	0.8	12.0	1.3	18	15	82
6	24	21.09	5,900	14	43	45	0.9	10.0	1.1	19	34	66
7	32	24.15	4,100	15	46	44	1.4	12.8	1.3	17	20	73
8	25	20.03	8,200	13.2	39	40	1.1	12.4	1.25	14	15	50
9	19	24.49	7,500	16.2	47	31	0.8	11.4	1.2	21	32	126
10	19	19.00	7,500	15.8	47	34	1.3	10.5	1.1	18	10	182
Mean	24.7	21.48	6,530	15	14	42	1	11	1	16	19	92
SD	4.3	2.57	1202.8	0.9	2.4	7.3	0.2	2.3	0.1	2.9	8.6	40.7

4.4 Pharmacokinetics

4.4.1 Ciprofloxacin pharmacokinetics

Phase 1: Pharmacokinetics of 500 mg ciprofloxacin orally twice daily for 7 days

After multiple oral ciprofloxacin dose of 500 mg alone in ten subjects, the mean values of C_{max}, AUC_{0-48}, $AUC_{0-\infty}$, $AUMC_{0-48}$, $AUMC_{0-\infty}$, MRT_{0-48}, $MRT_{0-\infty}$, λ_z, T_{max}, $T_{1/2,Z}$, V_z/f, and CL/f of ciprofloxacin were 3189.23 ± 831.28 ng/mL, 20140.25 ± 4256.29 ng.h/mL, 20949.47 ± 4220.93 ng.h/mL, 192055.22 ± 60277.32 $ng.h^2/mL$, 243819.94 ± 83710.40 $ng.h^2/mL$, 9.51 ± 1.84 h, 11.67 ± 3.66 h, 0.08 ± 0.02 h, 1.25 ± 0.68 h, 9.42 ± 2.49 h, 0.33 ± 0.12 L, and 0.02 ± 0.01 L/h, respectively (Table 5).

Phase 2: Pharmacokinetics of multiple oral dose of 500 mg ciprofloxacin and 200 mg itraconazole twice daily for 7 days

The mean values of C_{max}, AUC_{0-48}, $AUC_{0-\infty}$, $AUMC_{0-48}$, $AUMC_{0-\infty}$, MRT_{0-48}, $MRT_{0-\infty}$, λ_z, T_{max}, $T_{1/2,Z}$, V_z/f, and CL/f of ciprofloxacin administered in combination with itraconazole in ten subjects were 2995.82 ± 702.50 ng/mL, 21621.71 ± 4058.49 ng.h/mL, 22640.92 ± 3998.61 ng.h/mL, 210367.22 ± 49302.62 $ng.h^2/mL$, 275348.26 ± 71723.31 $ng/mL.h^2$, 9.69 ± 1.35 h, 12.15 ± 2.76 h, 0.07 ± 0.02 h, 1.90 ± 0.91 h, 9.93 ± 2.11 h, 0.328 ± 0.097 L, and 0.023 ± 0.004 L/h, respectively (Table 5).

All pharmacokinetic parameters of ciprofloxacin alone and in combination with itraconazole were not significantly different (p>0.05) (Table 5). The mean plasma ciprofloxacin concentration-time curves of two phases are shown in Figure 19. Individual plasma concentration-time curves of ciprofloxacin are shown in appendix D.

4.4.2 Itraconazole pharmacokinetics

Phase 1: Pharmacokinetics of 200 mg itraconazole orally twice daily for 7 days

After multiple oral dose of 200 mg itraconazole alone in ten subjects, the mean values of C_{max}, AUC_{0-96}, $AUC_{0-\infty}$, $AUMC_{0-96}$, $AUMC_{0-\infty}$, MRT_{0-96}, $MRT_{0-\infty}$, λ_z, T_{max}, $T_{1/2,Z}$, V_z/f, and CL/f of itraconazole were 1919.12 ± 986.93 ng/mL, 87958.73 ± 48460.66 ng.h/mL, 126016.10 ± 76391.96 ng.h/mL, 3287081.43 ± 1884772.44 $ng.h^2/mL$, 1086819 7.21 ± 9823024.22 $ng.h^2/mL$, 37.39 ± 4.18 h, 77.59 ± 35.78 h, 0.02 ± 0.01 h, 3.4±1.26 h, 53.13 ± 27.00 h, 0.17 ± 0.12 L/kg, and 0.0027 ± 0.0026 L/h, respectively (Table 6).

Phase 2: Pharmacokinetics of multiple oral dose of 200 mg itraconazole and 500 mg ciprofloxacin twice daily for 7 days

The mean values of C_{max}, AUC_{0-96}, $AUC_{0-\infty}$, $AUMC_{0-96}$, $AUMC_{0-\infty}$, MRT_{0-96}, $MRT_{0-\infty}$, λ_z, T_{max}, $T_{1/2,Z}$, V_z/f, and CL/f of itraconazole were 2937.65 ± 1501.86 ng/mL, 131236.29 ± 73746.13 ng.h/mL, 229937.39 ± 135339.64 ng.h/mL, 5023646.43 ± 2953079.03 $ng.h^2/mL$, 28199506.70 ± 23203908.82 $ng.h^2/mL$, 37.45 ± 6.04 h, 106.51 ± 59.38 h, 0.01 ± 0.01 h, 4.30 ± 2.67 h, 72.34 ± 41.14 h, 0.11 ± 0.08 L/kg, and 0.0015 ± 0.0012 L/h, respectively (Table 6).

The results demonstrated that ciprofloxacin when administered in combination with itraconazole significantly increased itraconazole's mean C_{max}, AUC_{0-96}, $AUC_{0-\infty}$, $AUMC_{0-96}$, and $AUMC_{0-\infty}$ compared with itraconazole alone by 53.07% (1919.12 ± 986.93 vs 2937.65 ± 1501.86), 49.20% (87958.73 ± 48460.66 vs 131236.29 ± 73746.13), 82.47% % (126016.10 ± 76391.96 vs 229937.39 ± 135339.64), 52.83% (3287081.43 ± 1884772.44 vs 5023646.43 ± 2953079.03), and 159.47% (1086197.21 ± 9823024.22 vs 28199506.70 ± 23203908.82), respectively, whereas V_z/f of itraconazole was significantly decreased by 34.27% (0.17 ± 0.12 vs 0.11 ± 0.08). However, ciprofloxacin did not affect other pharmacokinetic parameters of itraconazole (Table 6). The mean plasma itraconazole concentration-time curves of both phases are shown in Figure 20 and the individual plasma itraconazole concentration-time profiles are illustrated in appendix E.

Table 5. Pharmacokinetic parameters (mean ± SD) of ciprofloxacin in subjects after receiving multiple oral 500 mg ciprofloxacin alone and in combination with 200 mg itraconazole twice daily for 7 days.

Parameters	Ciprofloxacin concentration (ng/mL)		*P* value *t-test*
	alone	combination	
C_{max}(ng/mL)	3189.23 ± 831.28	2995.82 ± 702.50	NS
AUC_{0-48} (ng.h/mL)	20140.25 ± 4256.29	21621.71 ± 4058.49	NS
$AUC_{0-\infty}$ (ng.h/mL)	20949.47 ± 4220.93	22640.92 ± 3998.61	NS
$AUMC_{0-48}$ (ng.h^2/mL)	192055.22 ± 60277.32	210367.22 ± 49302.62	NS
$AUMC_{0-\infty}$ (ng.h^2/mL)	243819.94 ± 83710.40	275348.26 ± 71723.31	NS
MRT_{0-48} (h)	9.51 ± 1.84	9.69 ± 1.35	NS
$MRT_{0-\infty}$ (h)	11.67 ± 3.66	12.15 ± 2.76	NS
λ_z (h^{-1})	0.08 ± 0.02	0.07 ± 0.02	NS
$T_{1/2,z}$ (h)	9.42 ± 2.49	9.93 ± 2.11	NS
T_{max} (h)	1.25 ± 0.68	1.90 ± 0.91	NS
Cl/f (L/h)	0.02 ± 0.01	0.02 ± 0.004	NS
$V_{z/f}$ (L)	0.33 ± 0.12	0.33 ± 0.10	NS

NS: no significant difference between two treatments

Table 6. Pharmacokinetic parameters (mean ± SD) of itraconazole in subjects after receiving multiple oral 200 mg itraconazole alone and in combination with 500 mg ciprofloxacin twice daily for 7 days

Parameters	Itraconazole concentration (ng/mL)		*P* value *t-test*
	alone	combination	
C_{max}(ng/mL)	1919.12 ± 986.93	2937.65 ± 1501.86	*P*=0.018
AUC_{0-96} (ng.h/mL)	87958.73 ± 48460.66	131236.29 ± 73746.13	*P*=0.023
$AUC_{0-\infty}$ (ng.h/mL)	126016.10 ± 76391.96	229937.39 ± 135339.64	*P*=0.008
$AUMC_{0-96}$ (ng.h^2/mL)	3287081.43 ± 1884772.44	5023646.43 ± 2953079.03	*P*=0.020
$AUMC_{0-\infty}$ (ng.h^2/mL)	1086197.21 ± 9823024.22	28199506.70 ± 23203908.82	*P*=0.032
MRT_{0-96} (h)	37.93 ± 4.18	37.45 ± 6.04	NS
$MRT_{0-\infty}$ (h)	77.59 ± 35.78	106.51 ± 59.38	NS
λ_z (h^{-1})	0.002 ± 0.001	0.01 ± 0.01	NS
$T_{1/2,z}$ (h)	53.13 ± 27.00	72.34 ± 41.14	NS
T_{max} (h)	3.40 ± 1.26	4.30 ± 2.67	NS
Cl/f (L/h)	0.0027 ± 0.0026	0.0015 ± 0.0012	NS
$V_{z/f}$ (L)	0.17 ± 0.12	0.11 ± 0.08	*P*=0.041

NS: no significant difference between the two treatments

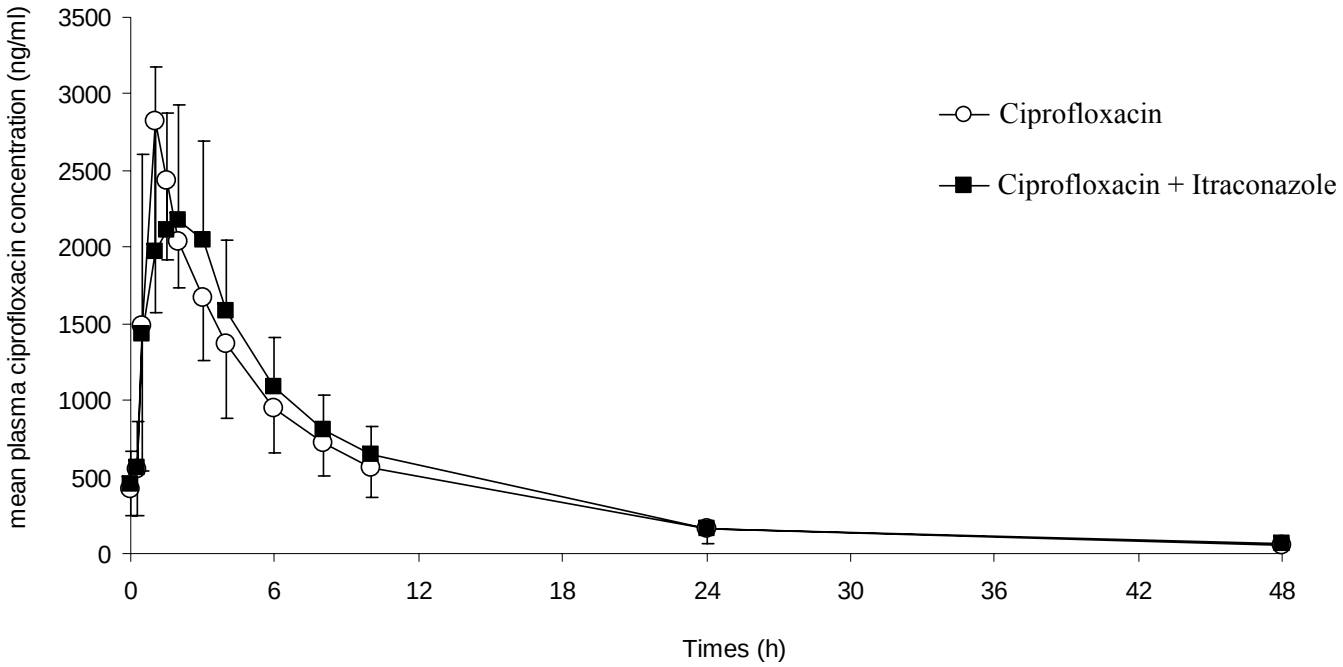


Figure 19. Mean plasma concentration-time curves of ciprofloxacin after multiple oral doses of ciprofloxacin alone and in combination with itraconazole twice daily for 7 days. -○- : multiple oral 500 mg ciprofloxacin alone twice daily for 7 days; -■- : multiple oral 500 mg ciprofloxacin in combination with 200 mg itraconazole twice daily for 7 days.

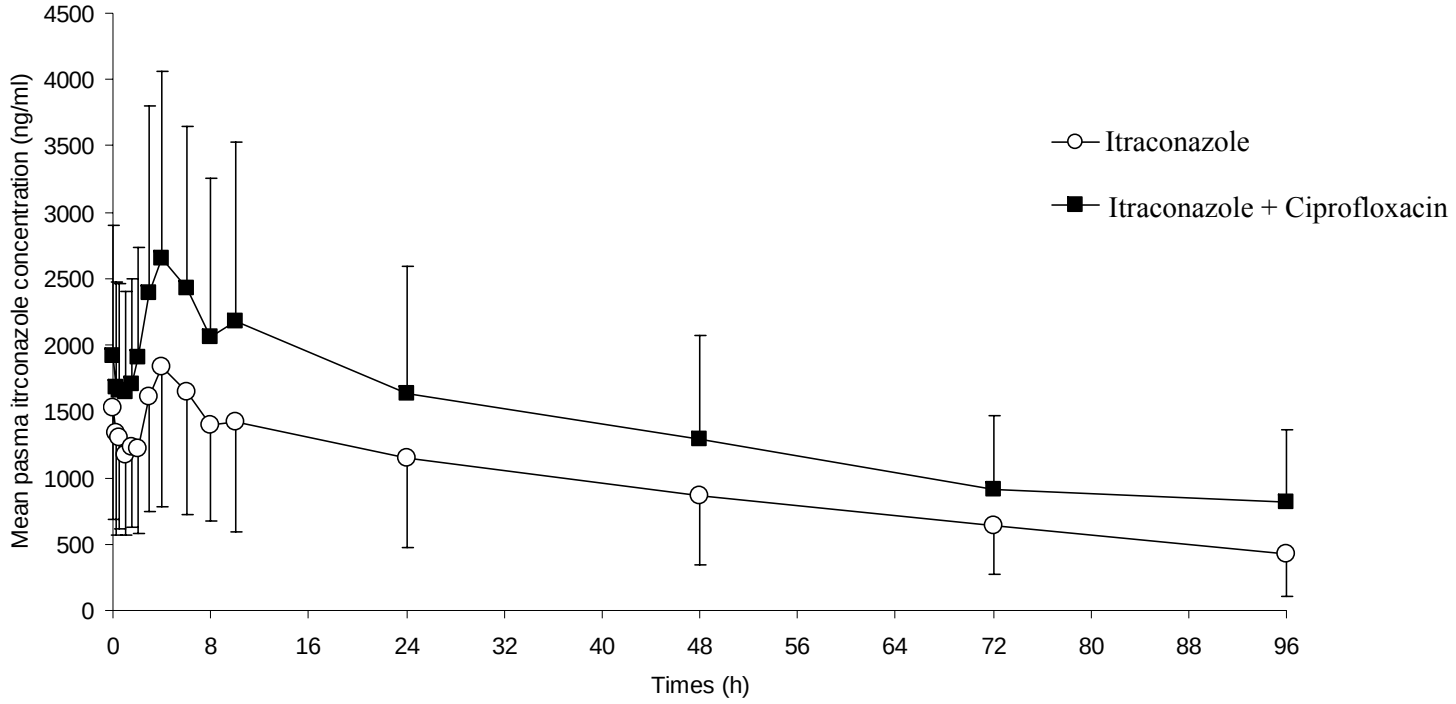


Figure 20. Mean plasma concentration-time curves of itraconazole after multiple oral doses of itraconazole alone and in combination with ciprofloxacin twice daily for 7 days. -○- : multiple oral 200 mg itraconazole alone twice daily for 7 days; -■- : multiple oral 200 mg itraconazole in combination with 500 mg ciprofloxacin twice daily for 7 days.

CHAPTER 5

DISCUSSION AND CONCLUSION

The results of the present study revealed that ciprofloxacin decreased the metabolism of itraconazole when they were administered concurrently for 7 days which was due most likely to inhibition of CYP isozyme CYP3A4. On the contrary, itraconazole did not alter the pharmacokinetics of ciprofloxacin.

The recommended doses of ciprofloxacin in the treatment of bacterial infection diseases are ranging between 200 to 1000 mg per day (Davis *et al*., 1996), whereas the recommended doses of itraconazole for treatment of systemic and superficial fungal infection are ranging between 200 to 600 mg per day (Grant and Clissold, 1989). In our study, ciprofloxacin was given to the healthy Thai male volunteers at the dose of 500 mg twice daily for 7 days, while itraconazole was given orally 200 mg twice daily for 7 days because these doses are sufficient to inhibit CYP3A4 isozymes as described by Hedaya et al. (2006) and Raaska et al. (2002). The results of the present study showed that the plasma concentration-time data of ciprofloxacin and itraconazole were fitted to noncompartment model because the variation of these pharmacokinetic parameters may be influenced by inter-individual variations (high value of SD) and environmental factors (eg. sex, races, diet, smoking, coffee and alcohol).

Ciprofloxacin has been reported to be a potent inhibitor of CYP1A2 and CYP3A4 in humans and rat microsomes (McLellan *et al*., 1996), and also to be a substrate of ABCG2 transporter (Alvarenz *et al*., 2008). It has been well documented that itraconazole is a potent inhibitor of CYP3A4 and also a substrate of CYP3A4 (Grant and Clissold, 1989). Furthermore, itraconazole may be a P-glycoprotein (P-gp) substrate as shown by its accumulated concentration in brain in mdr1a (-/-) mice deficient in P-gp (Venkatakrishnan *et al*., 2000). Itraconazole has also been reported to be a P-gp inhibitor (Zhang and Benet, 2001). In pharmacokinetic point of views, itraconazole and ketoconazole have been reported to have a variety of drug

interactions with several co-administered drugs of which their metabolism is mediated through CYP3A4. However, it has been documented that both itraconazole and ketoconazole significantly increase the C_{max} and AUC of ciprofloxacin but significantly reduce the CL of ciprofloxacin in mice. These results may be due to its inhibition of P-gp at renal tubular cells (Abou-Auda *et al*., 2008).

When multiple oral doses of ciprofloxacin were co-administered with itraconazole for 7 days, the means of C_{max}, AUC_{0-48}, $AUC_{0-\infty}$, and $t_{1/2}$ of ciprofloxacin were not significantly changed. These results were in contrast to the results of the study of Abou-Auda et al. (2008). Their results showed that concomittant administration of ketoconazole or itraconazole with ciprofloxacin leads to significant increases in C_{max}, $AUC_{0-\infty}$, and $T_{1/2}$ of ciprofloxacin. The differences of ciprofloxacin pharmacokinetics in human and mice may be explained by species different. In human, MDR1 is only P-gp conferring drug resistance and is of relevance to pharmacokinetics, whereas mice have two distinct proteins, MDR1a and MDR1b (Venkatakrishnan *et al*., 2000)].

Generally, the major metabolic pathways of gyrase inhibitors are piperazine ring-based reactions (formation of oxo-compounds, N-oxides, demethylation products where applicable, or ring cleavage with or without subsequent metabolic conversion) and acyl-glucuronidation at the carboxy group of the nucleus (Sorgel, 1989). The biotransformation of ciprofloxacin in *Gloeophyllum striatum*, the brown rot fungus, to at least 16 metabolites is mediated by hydroxylation (Wetzstein *et al*., 1999). On the other hand, the metabolism of ciprofloxacin in *Trichoderma viride*, a fungus and a biofungicide, to metabolites is by sulfate, formyl, and acetyl conjugation (Parshikov *et al*., 2002). The involvement of CYPs pathway has not been study with ciprofloxacin metabolism. However, ciprofloxacin is largely eliminated by renal excretion, and itraconazole is a very effective inhibitor of active tubular flux of many drugs (Jalava *et al*., 1997; Nishihara *et al*., 1999). The concurrent administration of ciprofloxacin with itraconazole therefore raises the possibility of pharmacokinetic drug interaction.

Ciprofloxacin is a dibasic compound (Barbosa *et al*., 2001). In the blood, it is mainly found as zwitterions (roughly 80%) which is one of electrically neutral forms of the molecule but which also behaves as anionic and cationic form

(Dautrey *et al*., 1999). A previous study demonstrated that ciprofloxacin intestinal elimination in rats seems to be mediated by organic anion and/or cation transporters (Dautrey *et al*., 1999). It is well known that ciprofloxacin is mainly eliminated by renal and nonrenal routes (Sorgel and Kinzing, 1993b). Thus, there is a possibility that ciprofloxacin excretion in human by OCT or OAT might have been occurred.

The results in the present study showed that all pharmacokinetic parameters of ciprofloxacin were not altered by itraconazole co-administration. These results could imply and suggest that CYP3A4 and P-gp do not play important roles in ciprofloxacin metabolism and excretion. Indeed, a previous study confirming that MDR1 does not contribute to ciprofloxacin secretory transport (Lowes and Simmons, 2002). Abou-Auda et al. (2008) recommended that ketoconazole or itraconazole should not be co-administered with ciprofloxacin, unless appropriate dose adjustment of ciprofloxacin is made. However, the dose adjustment of ciprofloxacin may not be necessary since pharmacokinetic parameters of ciprofloxacin in healthy volunteers in the present study were not altered by itraconazole co-administration.

When multiple oral dose of itraconazole was co-administered with ciprofloxacin, the mean C_{max}, $AUC_{0\text{-}96}$, and $AUC_{0\text{-}\infty}$ of itraconazole were significantly increased compared to itraconazole alone. The increase in C_{max} and AUC of itraconazole suggests that the metabolism of itraconazole might be decreased. McLellan et al. (1996) suggest that ciprofloxacin is a competitive inhibitor of CYP3A4 and CYP3A2 activity. Co-administration of ciprofloxacin with CYP3A4 substrates can result in clinically significant drug interactions. For example, the plasma concentration of clozapine is increased after administration of ciprofloxacin (Brouwers *et al*., 2009), whereas AUC and C_{max} of sildenafil are significantly increased by 110% and 117%, respectively in healthy volunteers who concurrently received a single oral dose of 500 mg ciprofloxacin (Hedaya *et al.,* 2006). Itraconazole is known to be metabolized by the set of cytochrome P450 isozymes including CYP3A4. Thus, co-administration of itraconazole with ciprofloxacin can theoretically lead to increasing in C_{max} and AUC of itraconazole and this notation is confirmed by the results of the present study.

Co-administration of itraconazole with ciprofloxacin resulted in the decrease of CL of itraconazole. These changes led to corresponding markedly

increased in C_{max} and AUC of itraconazole, suggesting that the metabolism of itraconazole was decreased. In theory, the $T_{1/2}$ and λ_z are known as dependent parameters because these values depend on the V_z/f and CL of the agent. The volume of distribution of a drug can be changed because of a change in clearance.

The V_z/f of itraconazole was significantly decreased when compared to itraconazole alone. The alteration in V_z/f of itraconazole led to corresponding decreased in CL, λ_z of itraconazole and the protein binding might be increased the free-drug of itraconazole in plasma because ciprofloxacin has a binding affinities to human serum albumin (HSA) was higher than itraconazole (log K′hsa = 0.14 and 1.04, respectively) (Colmenarejo *et al*., 2001). The mean T_{max} value of itraconazole was not significantly different when compared to itraconazole alone. Therefore, result in this study indicated that ciprofloxacin has no effect on the rate of itraconazole absorption.

Total clearance is defined by renal clearance and non-renal clearance. In previous study found that the C_{max} and $T_{1/2}$ of itraconazole did not significantly affected by renal dysfunction (Boelaert *et al*., 1988). Because of the lack of renal metabolism, the dose of itraconazole does not need to be reduced in patients with renal failure and supplementation after dialysis is not necessary (Beule and Gestel, 2001). In addition, itraconazole is extensively metabolized by liver, and the metabolites are eliminated through the bile and urine, but unchanged drug is not detected in the urine. Only 3 to 18% of the dose is detected in the feces (Beule and Gestel, 2001). Theoretically, if a drug is metabolized exclusively by liver, the CL/f of the drug is equal to the hepatic clearance (CL_H). Hepatic clearance is defined as the volume of blood perfusing the liver that is cleared of drug per unit of time. Calculation of hepatic clearance is based on total hepatic blood flow (Q_H), fraction unbound of drug in the blood (f_u) and the free intrinsic clearance (CL_{int}): $CL_H = Q_H \times f_u \times CL_{int} / Q_H + f_u \times CL_{int}$. In addition, the plasma drug concentration data can also be obtained from the equation: hepatic clearance (CL_H) = (rate of elimination by liver) / plasma drug concentration). The CL_{int} was decreased in ciprofloxacin combination since a major part of these parameters is metabolism. It is important to note that the pharmacokinetic consequences of enzyme inhibition should always be an increase in plasma concentrations, because cytochrome P450 inhibition will cause a decrease in

the hepatic and intestinal metabolism. Accordingly, ciprofloxacin decreased hepatic clearance of itraconazole because ciprofloxacin is an inhibitor of human liver microsomal CYP3A4 activity and itraconazole undergoes mainly metabolism by the set of cytochrome P450 isozymes.

The mean $T_{1/2}$ of itraconazole was prolonged but not significant when compared to itraconazole alone, which was thought to be due to decrease in CL of itraconazole. However, the $T_{1/2}$ of itraconazole was increased in 3 subjects when compared to itraconazole alone. It could be explained as an individual variation and may be due to CYP3A4 polymorphism. The CYP3A4 activity varies widely in human, and more than 78 DNA sequence polymorphisms are known (Keshava *et al*., 2004). In addition, at steady state the terminal $T_{1/2}$ increases to 30 hours, indicating that the itraconazole elimination mechanism is saturated at clinical doses (Grant and Clissold, 1989).

Itraconazole is considered to be both more effective and safer than ketoconazole, and most of the adverse reactions are dose-related. Gastrointestinal disorders are the most common adverse effects (nausea, vomiting, diarrhea and dry mouth), cutaneous side-effects (eruption and pruritus), neurologic side effects (headache, dizziness and vertigo) and hepatic side effects (Amichai and Grunwald, 1998). In our study, the adverse effects were observed when ciprofloxacin and itraconazole were concurrently administered. Nausea (60%), dry mouth (40%), diarrhea (10%) and headache (10%) were noted after co-administration of itraconazole and ciprofloxacin. These adverse effects might be due to the fact that ciprofloxacin increases the C_{max} and AUC of itraconazole. However, any adverse effects after administration of ciprofloxacin or itraconazole alone were not observed.

The effect of ciprofloxacin on the pharmacokinetics of itraconazole is probably due to the CYP inhibition of ciprofloxacin because itraconazole metabolism is mediated by CYP, especially the CYP3A4 isoform (Isoherranen *et al*., 2004). The CYP3A4 is the most abundant isoform, accounting for about 30% of total CYP in the liver (Federico and Mario, 2001). However, this interaction may be caused by inhibition of other CYP450 isoforms by ciprofloxacin, but there is no evidence to support this notion. On the contrary, the effect of itraconazole on the

pharmacokinetics of ciprofloxacin was not observed since ciprofloxacin is neither a substrates of CYP3A4 nor P-gp as previous described.

In conclusion, this study demonstrates that ciprofloxacin markedly increases the plasma concentration and AUC of itraconazole. These effects may be due to mainly inhibition of CYP3A4 isozyme by ciprofloxacin. However, there is no significant effect of itraconazole on the metabolism of ciprofloxacin. Therefore, the dosage of itraconazole should be reduced and its therapeutic outcome be closely monitored when these two agents are concomitantly administered, especially in patients who will be on a long-term therapy because the incidence of adverse effects will be higher.

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APPENDIX

APPENDIX A

Laboratory values of Liver and Renal Function

Parameters	Normal Values
Aspatate aminotransferase (AST, SGOT)	0-35 units/L
Alanine aminotransferase (ALT, SGPT)	0-35 units/L
Alkaline phosphatase	41-133 units/L
Total bilirubin	0.1-1.2 mg/dL
- direct bilirubin	0.1-0.4 mg/dL
Protein, total	6.0-8.0 g/dL
- albumin	3.4-4.7 g/dL
Creatinine	0.6-1.2 mg/dL

Reference: Nicoll CD. Therapeutic drug monitering & laboratory reference ranges In: Current medical diagnosis & treatment 2000, 39th ed, Tierney LM, McPhee SJ, Papadakis MA, eds, San Francisco, McGraw-Hill companies, 1671-25.

APPENDIX B

ใบยินยอมเข้าร่วมในโครงการ
ปฏิกิริยาต่อกันทางเภสัชจลนศาสตร์ระหว่างยาซิพโปรฟลอกซาซินกับไอทราโคนาโซล

ก่อนที่จะลงนามในใบยินยอมร่วมในการทำวิจัยนี้ ข้าพเจ้า (นาย, นาง, นางสาว……..นามสกุล.....ได้รับคำอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตราย หรืออาการที่อาจเกิดขึ้นจากการวิจัย หรือจากยาที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว ข้าพเจ้ายังได้รับสำเนาของหนังสือฉบับนี้ รวมทั้งเอกสารแนบท้ายเชิญชวนเข้าร่วมโครงการ 1 ชุด และได้อ่านข้อความข้างต้นทั้งหมดแล้ว

ผู้วิจัยรับรองว่าจะตอบคำถามต่างๆที่ข้าพเจ้าสงสัยด้วยความเต็มใจ ไม่ปิดบังซ่อนเร้น จนข้าพเจ้าพอใจ

ในระหว่างร่วมโครงการ หากการกระทำและคำชี้แจงของผู้วิจัยยังไม่เป็นที่เข้าใจ ข้าพเจ้ามีสิทธิ์แจ้งต่อประธานอนุกรรมการจริยธรรมของคณะวิทยาศาสตร์ได้ (รองคณบดีฝ่ายวิจัยและบัณฑิตศึกษา โทร. (047) 446660) และหากข้าพเจ้าไม่พอใจในการเข้าร่วมในโครงการ ข้าพเจ้ามีสิทธิ์ปฏิเสธการเข้าร่วมในโครงการได้ทันที โดยไม่เสียสิทธิ์ในการรับการรักษาในโรงพยาบาลสงขลานครินทร์ต่อไป

.....	.....
(ลายเซ็นของผู้ยินยอม)	วัน เดือน ปี
.....	.....
(ลายเซ็นของพยาน)	วัน เดือน ปี
……..	.....
(ลายเซ็นของแพทย์)	วัน เดือน ปี

เอกสารเชิญชวนเข้าร่วมในโครงการ

ชื่อเรื่อง **ปฏิกิริยาต่อกันทางเภสัชจลนศาสตร์ระหว่างยาซิพโปรฟลอกซาซินกับไอทราโคนาโซล**

เรียน ผู้อ่านที่นับถือ

พวกเราคณะผู้วิจัยใคร่ขอเล่าถึงโครงการวิจัยที่กำลังทำอยู่ และขอเชิญชวนท่านซึ่งเป็นผู้มีสุขภาพปกติเข้าร่วมโครงการนี้

เอกสารนี้จะให้ข้อมูลเพื่อช่วยให้ท่านตัดสินใจว่าจะเข้าร่วมโครงการนี้หรือไม่ ท่านควรจะได้เข้าใจรายละเอียดที่เกี่ยวข้องทั้งหมดก่อนที่ท่านจะตกลงเข้าร่วมการศึกษานี้ หากท่านยังมีคำถามที่ยังไม่ได้รับการอธิบายโดยละเอียดในเอกสารนี้ โปรดสอบถามแพทย์ที่ร่วมทำการศึกษานี้ได้ ท่านไม่ควรตกลงเข้าร่วมโครงการหากยังไม่เข้าใจวิธีการที่เกี่ยวข้องอย่างละเอียดดี

ลักษณะและวัตถุประสงค์ของการศึกษา

โครงการนี้เป็นการศึกษาเปรียบเทียบทางด้านเภสัชจลนศาสตร์เพื่อศึกษาถึงปฏิกิริยาต่อกันทางเภสัชจลนศาสตร์ระหว่างยาซิพโปรฟลอกซาซินกับไอทราโคนาโซล ซึ่งยาทั้งสองนี้จะบริหารร่วมกันในผู้ป่วยติดเชื้อราและเชื้อแบคทีเรียบางชนิด แพทย์ผู้ทำการศึกษาวิจัยจะขอให้ท่านพิจารณาเข้าร่วมในโครงการนี้ และจะมีอาสาสมัครสุขภาพปกติเข้าร่วมในโครงการนี้ 10 ราย โดยที่การศึกษานี้จะแสดงให้เห็นถึงอิทธิพลของยาซิพโปรฟลอกซาซินต่อเภสัชจลนศาสตร์ของยาไอทราโคนาโซล และอิทธิพลของยาไอทราโคนาโซลต่อเภสัชจลนศาสตร์ของยาซิพโปรฟลอกซาซินในอาสาสมัครสุขภาพปกติว่ามีมากน้อยเพียงใด ซึ่งจะมีผลต่อการกำหนดเป็นแนวทางในการบริหารยาทั้งสองร่วมกันในผู้ป่วยที่จำเป็นต้องได้รับการรักษาด้วยยาทั้งสองต่อไป

วิธีการ

การศึกษานี้จะเริ่มด้วยการคัดเลือกอาสาสมัครเข้าร่วมในโครงการแพทย์ผู้ทำการศึกษาจะซักประวัติทางการแพทย์ ตรวจร่างกาย ตรวจทางห้องปฏิบัติการ ซึ่งได้แก่ การตรวจนับเม็ดเลือดสมบูรณ์ หน้าที่การทำงานของตับและไต และตรวจการตั้งครรภ์ ซึ่งเป็นการตรวจปกติที่แพทย์จะต้องทำเพื่อยืนยันว่าสุขภาพของอาสาสมัครเป็นปกติ

อาสาสมัครที่ได้รับการคัดเลือกทุกคนจะต้องเข้าร่วมโครงการทั้งหมด 2 ระยะ

- ระยะที่ 1 ท่านจะได้รับยาซิพโปรฟลอกซาซิน ขนาด 500 มิลลิกรัม (1 เม็ด) ตามด้วยน้ำ 200 มิลลิลิตร หลังอาหารเวลา 08.00 นาฬิกา และเวลา 20.00 นาฬิกา นาน 7 วัน หรือไอทราโคนาโซล ขนาด 200 มิลลิกรัม (2 แคปซูล) ตามด้วยน้ำ 200 มิลลิลิตร หลังอาหารเวลา 08.00 นาฬิกา และเวลา 20.00 นาฬิกา นาน 7 วัน ชนิดใดชนิดหนึ่งก่อนหรือหลังกัน โดยวิธีการสุ่ม ในวันที่ 7 ของการได้รับยาในระยะที่ 1 ของการทดลอง ท่านต้องมาพร้อมกันที่ภาควิชาเภสัชวิทยา เวลา 07.00 น. ท่านต้องได้รับการคาสายพลาสติกไว้ในหลอดเลือดดำที่แขน (คา heparin lock) พร้อมทั้งรับประทานอาหารและเครื่องดื่มที่ผู้วิจัยจัดเตรียมให้ หลังจากนั้นจะได้รับยาซิพโปรฟลอกซาซิน ขนาด 500 มิลลิกรัม (1 เม็ด) หรือไอทราโคนาโซล ขนาด 200 มิลลิกรัม (2 แคปซูล) ตามด้วยน้ำ 200 มิลลิลิตร หลังอาหารเวลา 08.00 นาฬิกา และจะต้องถูกเก็บตัวอย่างเลือดจำนวน 5 มิลลิลิตร (1 ช้อนชา) ผ่านทาง heparin lock ซึ่งที่เวลา 0 (ก่อนบริหารยา), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 24, 72 และ 96 ชั่วโมงหลังบริหารยาในวันที่ 7 รวมทั้งหมด 15 ครั้ง แล้วนำเลือดที่เก็บได้ไปวัดความเข้มข้นของยา หลังจากนั้นท่านต้องทำการทดลองซ้ำเช่นเดิมอีกครั้งหนึ่งด้วยการรับประทานยาอีกชนิดหนึ่งที่ไม่ซ้ำกับครั้งแรก

- ระยะที่ 2 หลังจากระยะที่ 1 เป็นเวลา 14 วัน ท่านจะได้รับยาซิพโปรฟลอกซาซินพร้อมกับยาไอทราโคนาโซลที่เวลา 08.00 นาฬิกา และเวลา 20.00 นาฬิกา ขนาดยาและวิธีการบริหารยาทั้งสองจะทำเหมือนระยะที่ 1 เป็นเวลานาน 7 วัน ในวันที่ 7 ของการได้รับยาในระยะที่ 2 ของการทดลอง ท่านจะต้องปฏิบัติเช่นเดียวกับในระยะที่ 1 เพื่อนำเลือดไปวิเคราะห์ระดับยาต่อไป

สรุปรวมการศึกษาทั้ง 2 ระยะจะต้องใช้เวลานาน 7 สัปดาห์และมีการเจาะเลือดทั้งหมด 45 ครั้ง ในระหว่างทำศึกษา ท่านจะถูกซักประวัติและตรวจร่างกาย รวมทั้งการตรวจทางห้องปฏิบัติการที่จำเป็น เพื่อเฝ้าระวังผลข้างเคียงของยาที่อาจเกิดขึ้นได้ ถ้าเกิดผลข้างเคียงของยาที่รุนแรงจะหยุดการศึกษาทันที และผู้ป่วยจะได้รับการดูแลโดยแพทย์ในโครงการอย่างเหมาะสม

การทดลองนี้มีความเสี่ยงอะไรบ้าง

การเจาะเลือดจะใช้วิธีเก็บจากสายที่คาไว้ในหลอดเลือดดำ ดังนั้นท่านจะเจ็บครั้งแรกตอนเจาะเลือดเพื่อคาสายไว้ในหลอดเลือดดำเท่านั้น หลังจากนั้นเวลาเก็บเลือดแต่ละ

ครั้งจะไม่เจ็บ การคาสายไว้ในหลอดเลือดดำจะเกิดผลแทรกซ้อนน้อยมาก ได้แก่การอักเสบของหลอดเลือดดำเป็นต้น

ส่วนยาที่ท่านได้รับเป็นยาที่ใช้ในการรักษามาตรฐาน โดยยาซิพโปรฟลอกซาซินเป็นยาที่อาจเกิดผลข้างเคียงบ้าง ได้แก่ คลื่นไส้ อาเจียน ท้องเสีย พบได้ร้อยละ 2-8 และปวดศีรษะ มึนงง พบได้ร้อยละ 0.4-4.4 เป็นต้น ส่วนยาไอทราโคนาโซลอาจทำให้เกิดผลข้างเคียงบ้าง ได้แก่ คลื่นไส้ อาเจียน พบได้ร้อยละ 2 และปวดศีรษะ มึนงง พบได้ร้อยละ 1 เป็นต้น

ประโยชน์ที่คาดว่าจะได้รับ

การเข้าร่วมในโครงการนี้ท่านจะไม่ต้องเสียค่าใช้จ่ายในการตรวจเลือด ปัสสาวะ และตรวจร่างกาย นอกจากนั้น ในระหว่างทำการศึกษา ท่านจะได้รับการดูแลเป็นอย่างดีโดยแพทย์ผู้มีประสบการณ์ด้านนี้โดยเฉพาะ และประการสุดท้ายที่สำคัญคือ ข้อมูลที่ได้จากการเข้าร่วมในการทดลองของท่านจะเป็นประโยชน์อย่างมหาศาลต่อการกำหนดแนวทางในการบริหารยาซิพโปรฟลอกซาซินและไอทราโคนาโซลร่วมกัน

นอกจากนี้ท่านจะได้รับค่าตอบแทนในการเข้าร่วมโครงการวิจัยนี้เป็นเงิน 3,000 บาท (สามพันบาทถ้วน) เมื่อเข้าร่วมโครงการจนครบตามระยะที่กำหนด

ผู้ที่ท่านติดต่อ

หากท่านมีคำถามเพิ่มเติมเกี่ยวกับการศึกษาวิจัยนี้ หรือต้องการสอบถามเกี่ยวกับสิทธิในฐานะผู้เข้าร่วมในการศึกษา ท่านสามารถติดต่อกับนายแพทย์วิบูลย์ ฤทธิทิศ ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ โทรศัพท์ (047) 288180 หรือ 081-7665397 หรือนายแพทย์วีรวัฒน์ มหัทธนตระกูล ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ โทรศัพท์ (047) 288174 หรือ 081-9749089

การเก็บข้อมูลเป็นความลับ

ข้อมูลทุกอย่างที่ได้จากการผลทดลอง อันเนื่องมาจากการเข้าร่วมการศึกษาครั้งนี้จะถูกเก็บเป็นความลับอย่างเคร่งครัด ท่านจะได้รับแจ้งข้อมูลที่มีความสำคัญต่อสุขภาพของท่าน แต่จะไม่มีการเปิดเผยข้อมูลดังกล่าวต่อบุคคลที่ 3 ผลจากการศึกษาทั้งหมดจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุปผลการวิจัย

APPENDIX C

วท-จธ/51/12-1

ที่ ศธ 0521.1.09/ ๒๕๗

คณะวิทยาศาสตร์
มหาวิทยาลัยสงขลานครินทร์
ตู้ ปณ 3 คอหงส์ 90112

หนังสือรับรองโครงการวิจัย

การศึกษาวิจัยที่ทำการทดลองในมนุษย์เรื่อง เรื่อง "ปฏิกิริยาต่อกันทางเภสัชจลนศาสตร์ระหว่าง ยาซิพโปรฟลอกซาซินกับไอทราโคนาโซลในอาสาสมัครสุขภาพปกติ"

หัวหน้าโครงการวิจัย : อาจารย์สมชาย ศรีวิริยะจันทร์
ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์

ได้ผ่านการพิจารณาและเห็นชอบจาก คณะกรรมการจริยธรรมการวิจัยที่ทดลองในมนุษย์ คณะวิทยาศาสตร์

ให้ไว้ ณ วันที่ 3 มิถุนายน 2551

(รองศาสตราจารย์ ดร.วิไลวรรณ โชติเกียรติ)
รองคณบดีฝ่ายวิจัยและบัณฑิตศึกษา ปฏิบัติราชการแทน
คณบดีคณะวิทยาศาสตร์

APPENDIX D

Profile of plasma ciprofloxacin concentration at each time of blood drawn from individual subject

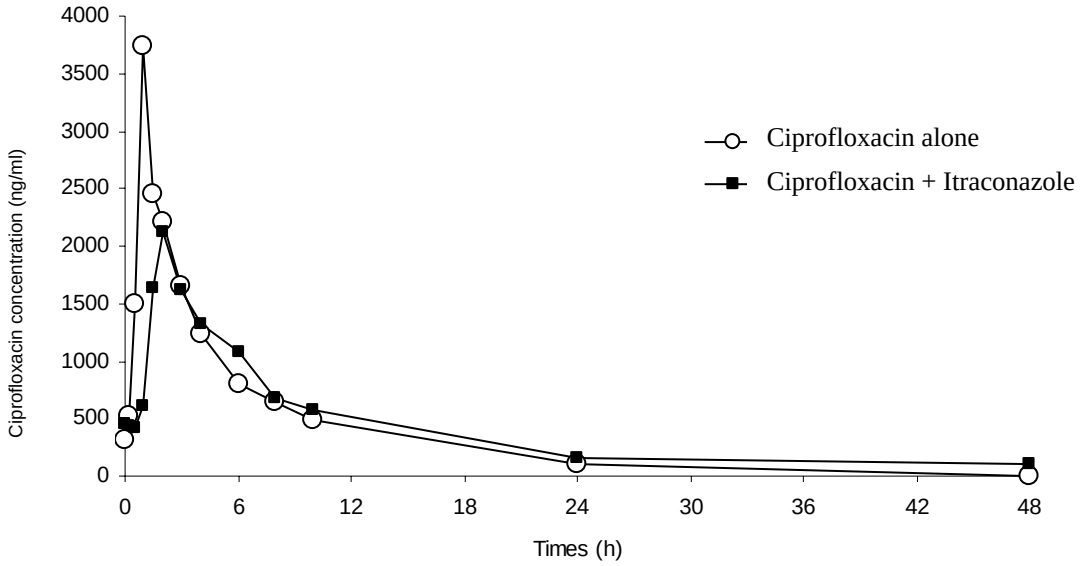

Figure 21. Plasma ciprofloxacin concentration-time curve of subject No. 1

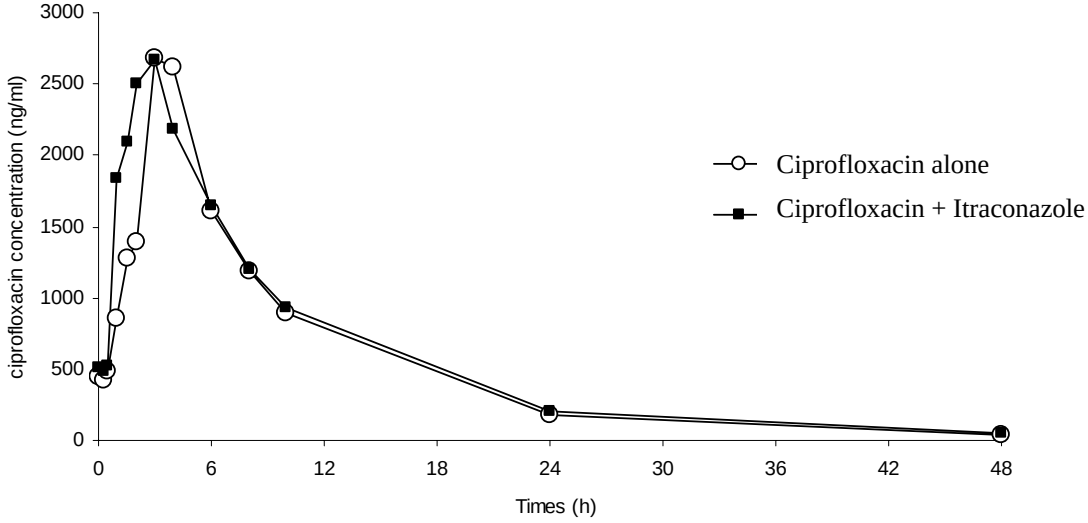

Figure 22. Plasma ciprofloxacin concentration-time curve of subject No. 2

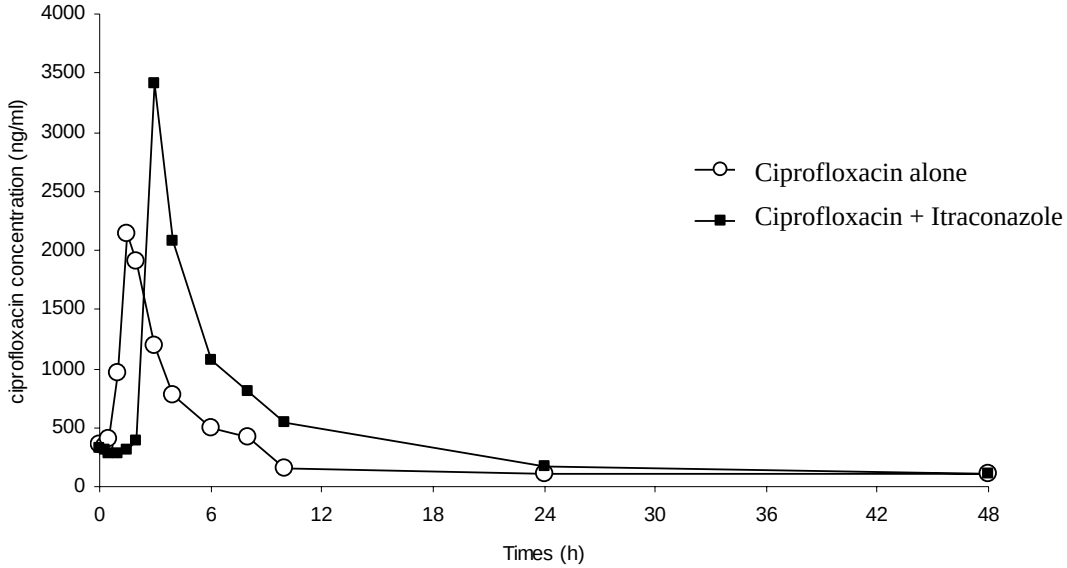

Figure 23. Plasma ciprofloxacin concentration-time curve of subject No. 3

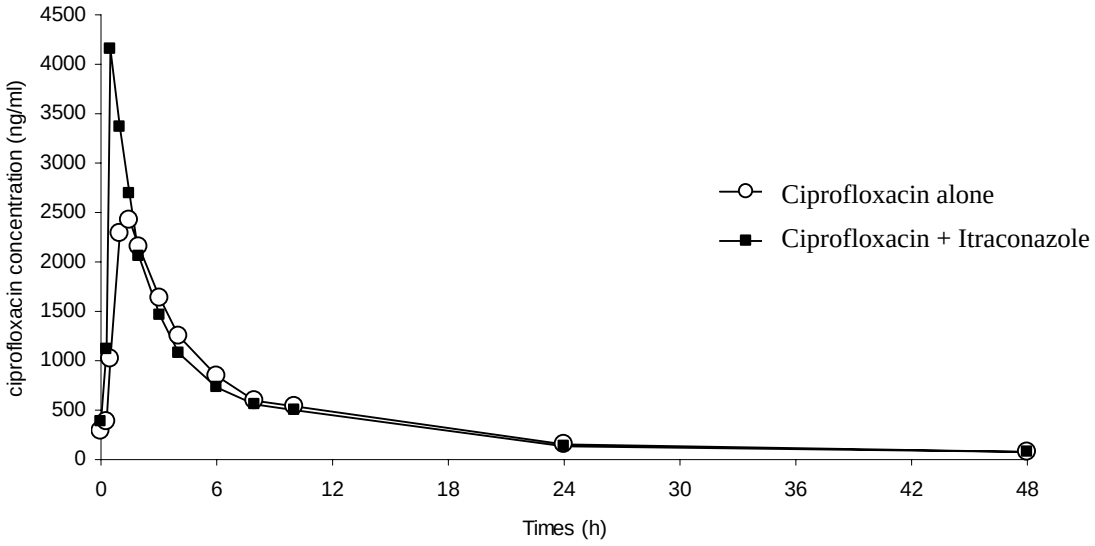

Figure 24. Plasma ciprofloxacin concentration-time curve of subject No. 4

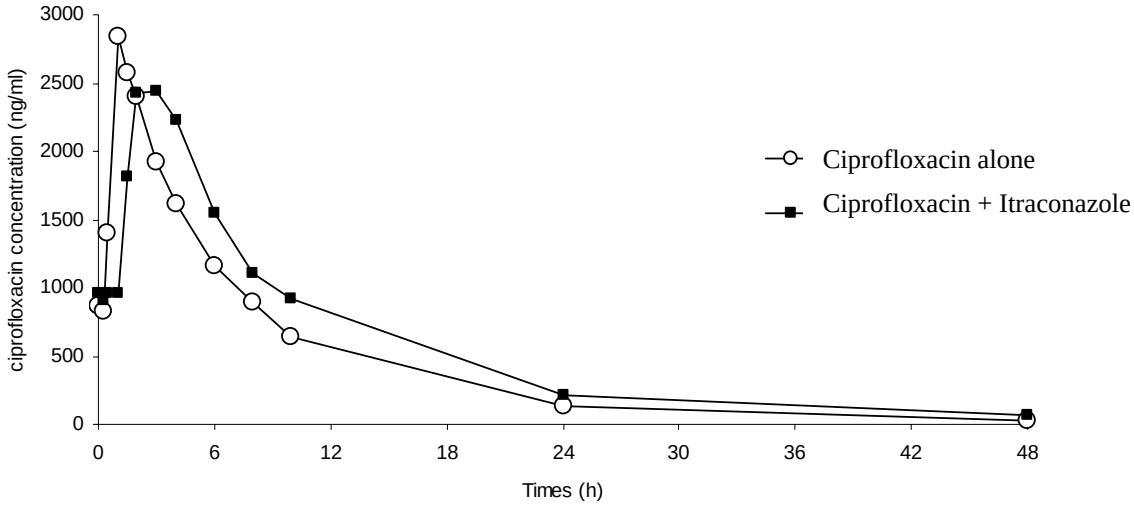

Figure 25. Plasma ciprofloxacin concentration-time curve of subject No. 5

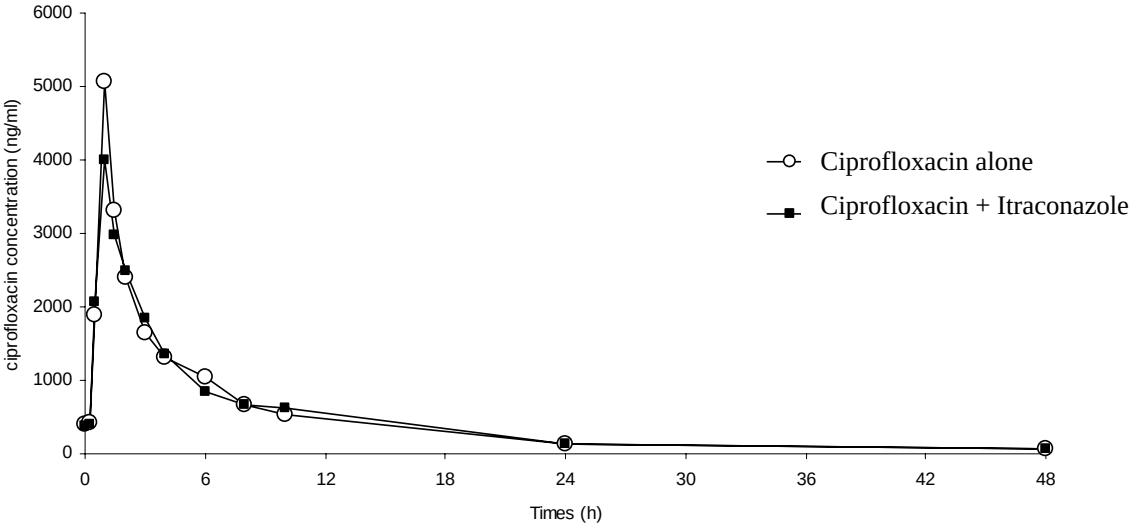

Figure 26. Plasma ciprofloxacin concentration-time curve of subject No. 6

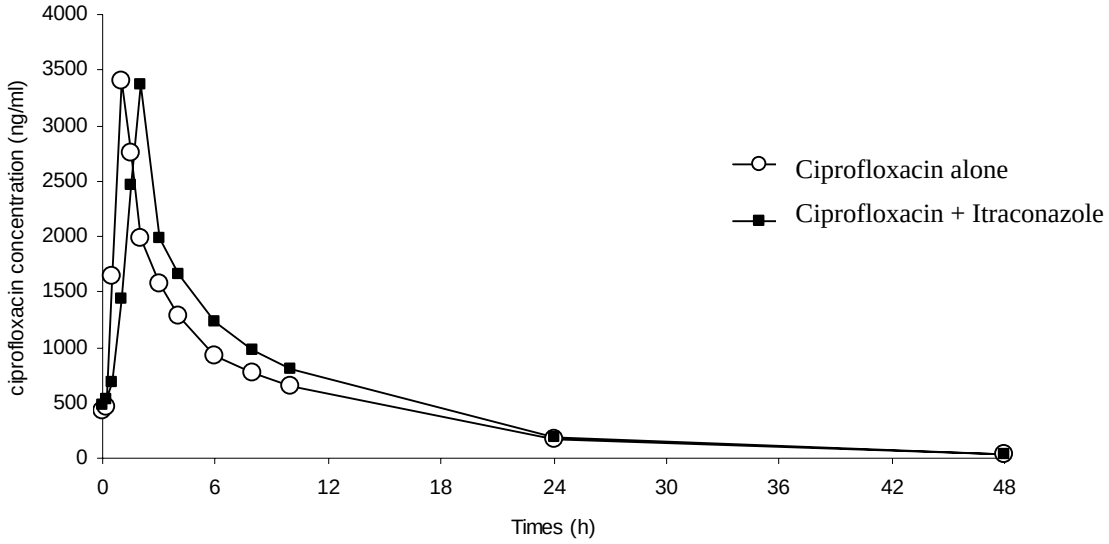

Figure 27. Plasma ciprofloxacin concentration-time curve of subject No. 7

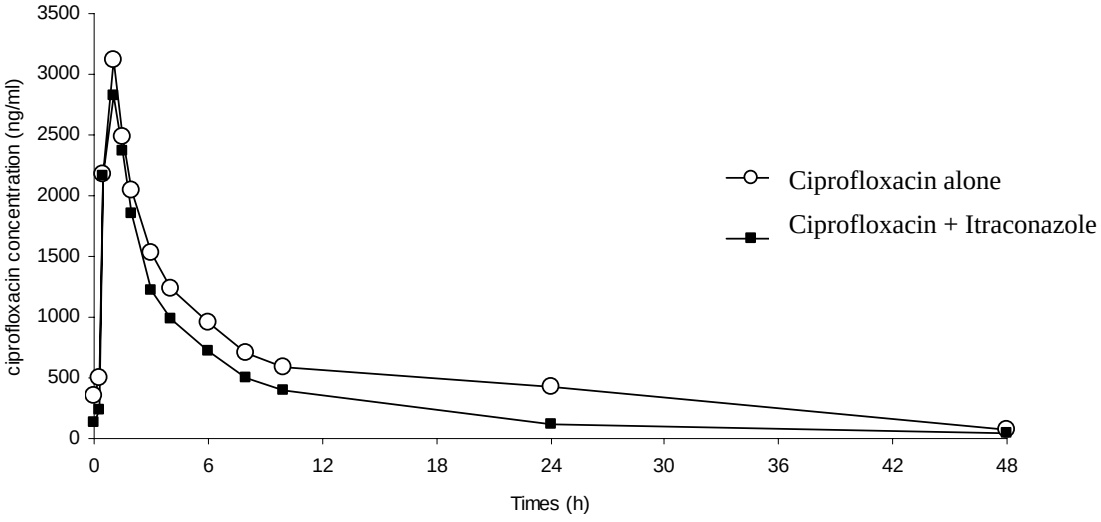

Figure 28. Plasma ciprofloxacin concentration-time curve of subject No. 8

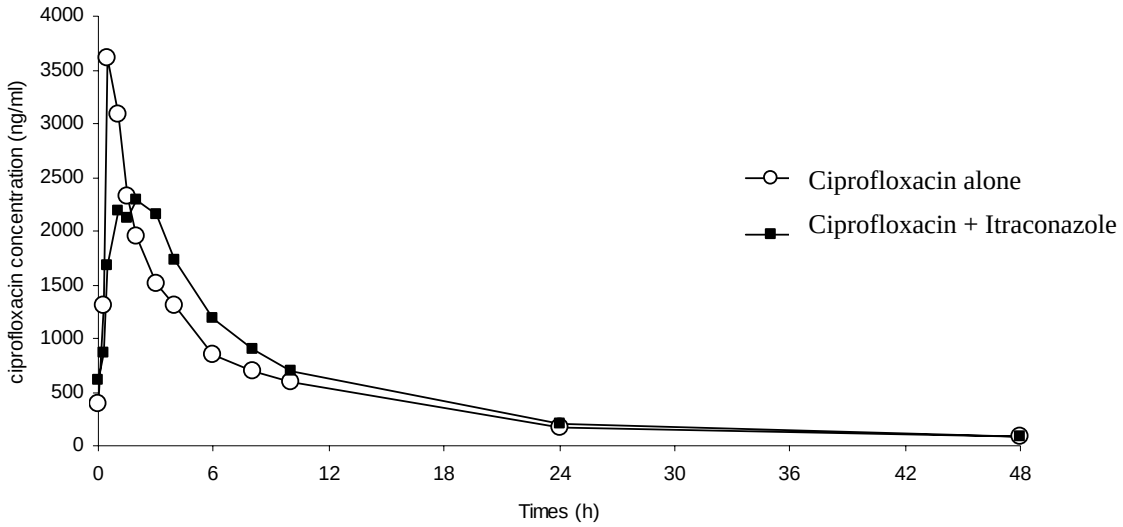

Figure 29. Plasma ciprofloxacin concentration-time curve of subject No. 9

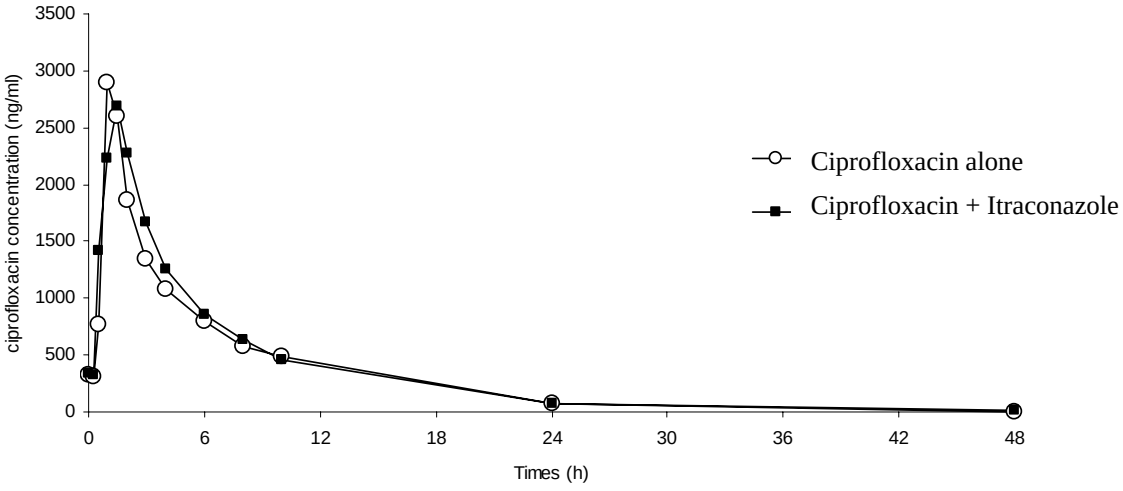

Figure 30. Plasma ciprofloxacin concentration-time curve of subject No. 10

APPENDIX E

Profile of plasma itraconazole concentration at each time of blood drawn from individual subject

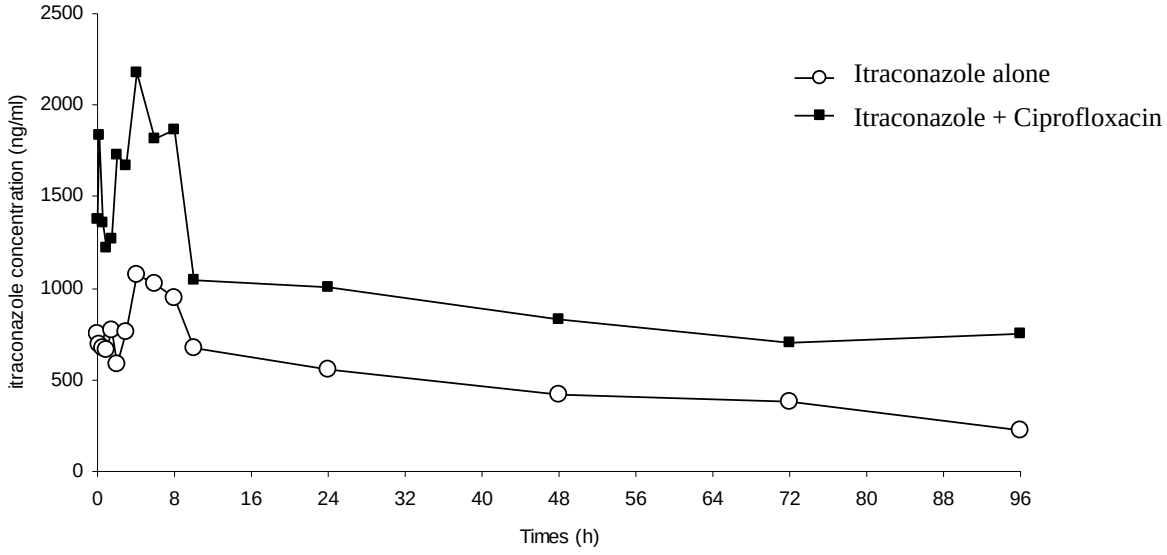

Figure 31. Plasma itraconazole concentration-time curve of subject No. 1

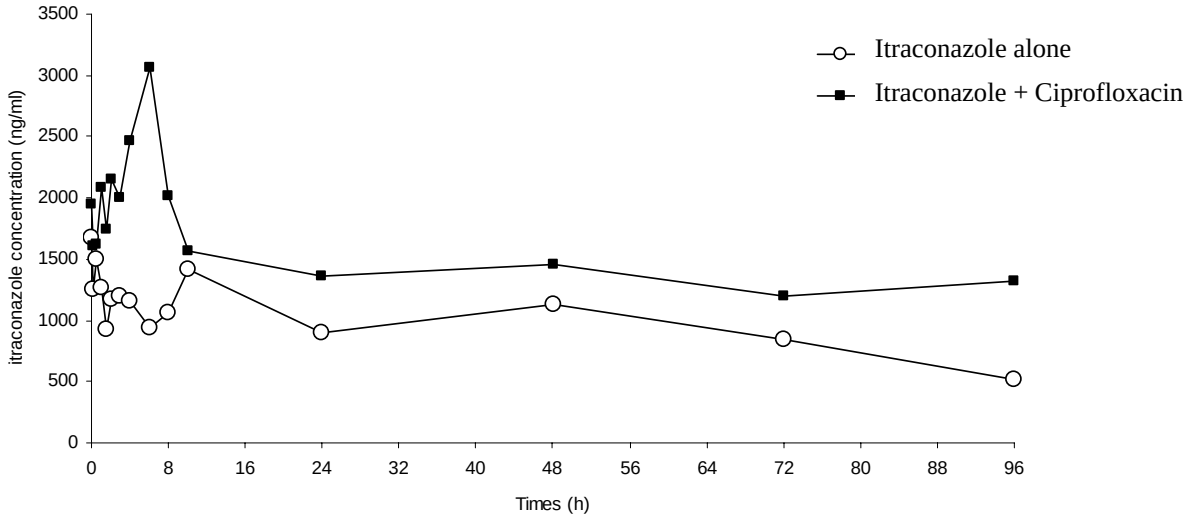

Figure 32. Plasma itraconazole concentration-time curve of subject No. 2

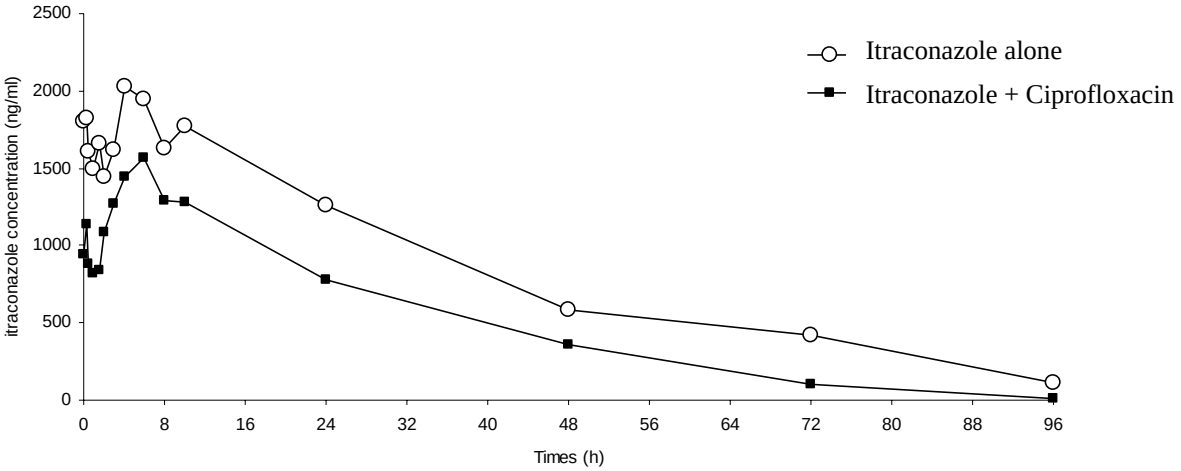

Figure 33. Plasma itraconazole concentration-time curve of subject No. 3

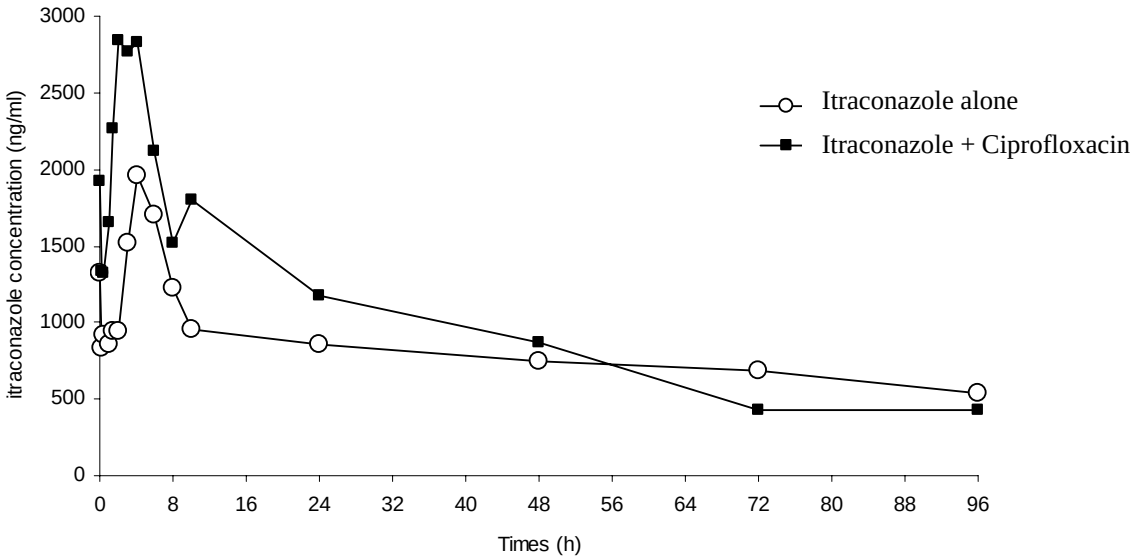

Figure 34. Plasma itraconazole concentration-time curve of subject No. 4

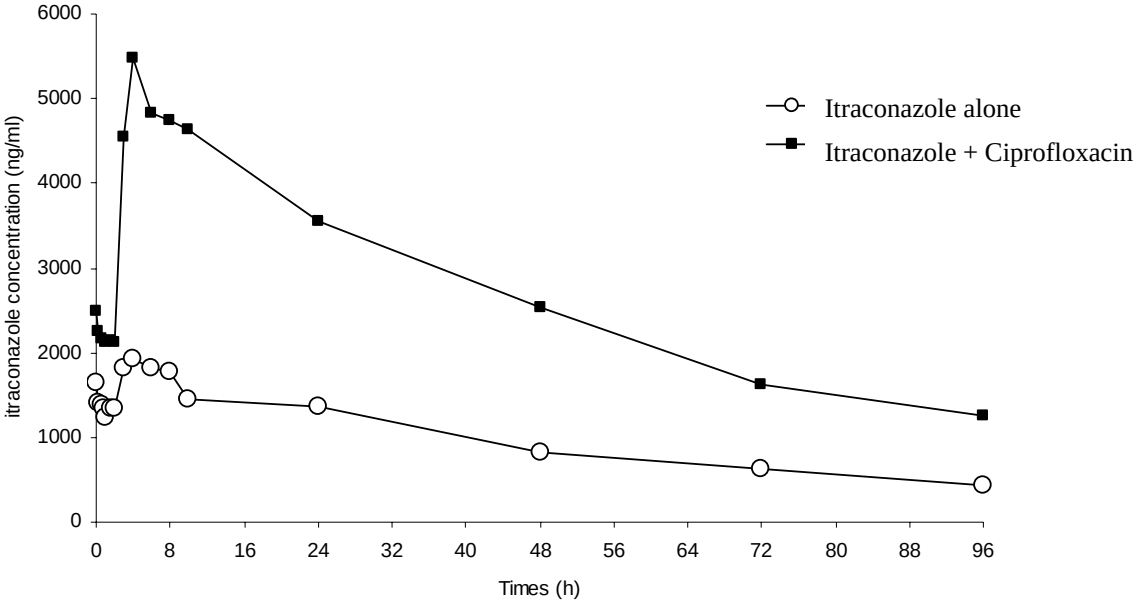

Figure 35. Plasma itraconazole concentration-time curve of subject No. 5

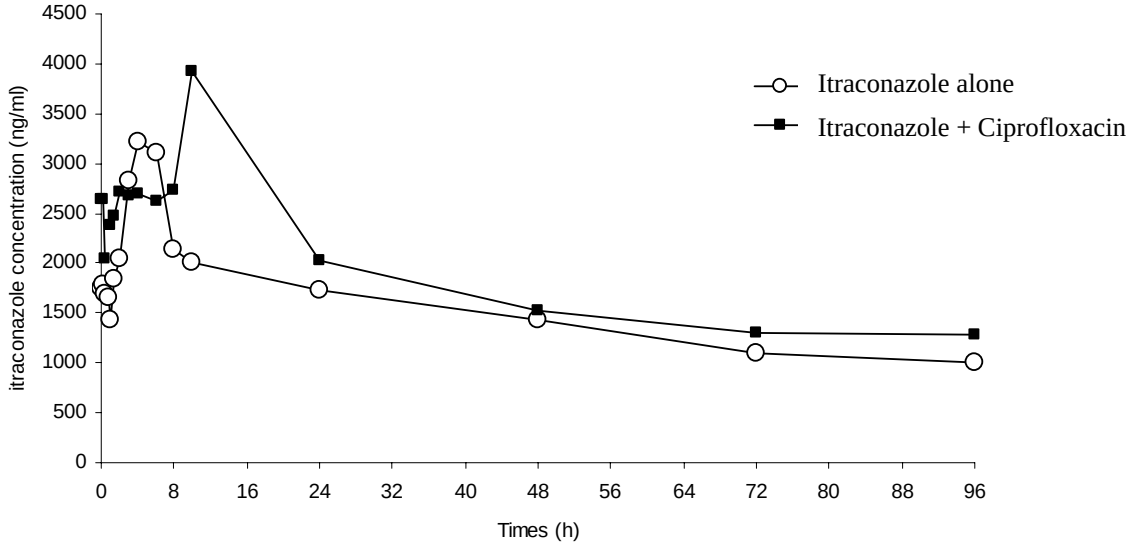

Figure 36. Plasma itraconazole concentration-time curve of subject No. 6

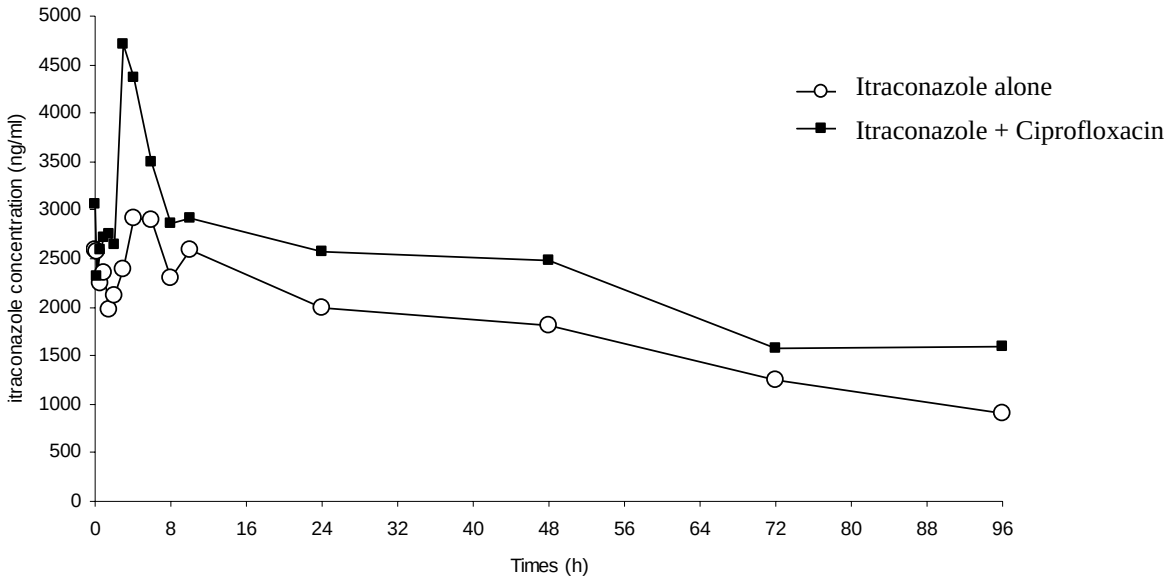

Figure 34. Plasma itraconazole concentration-time curve of subject No. 7

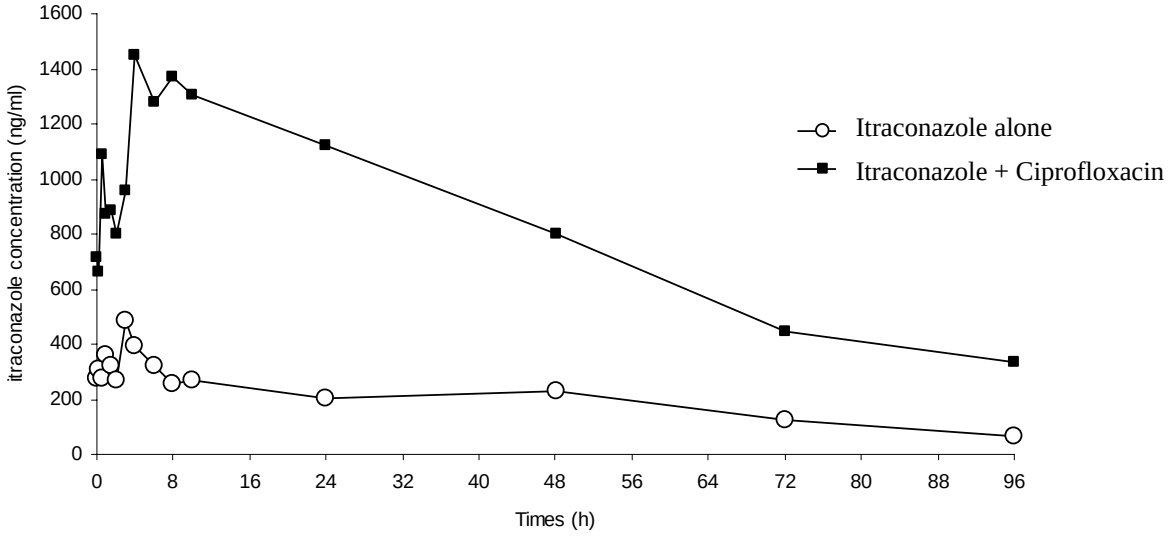

Figure 38. Plasma itraconazole concentration-time curve of subject No. 8

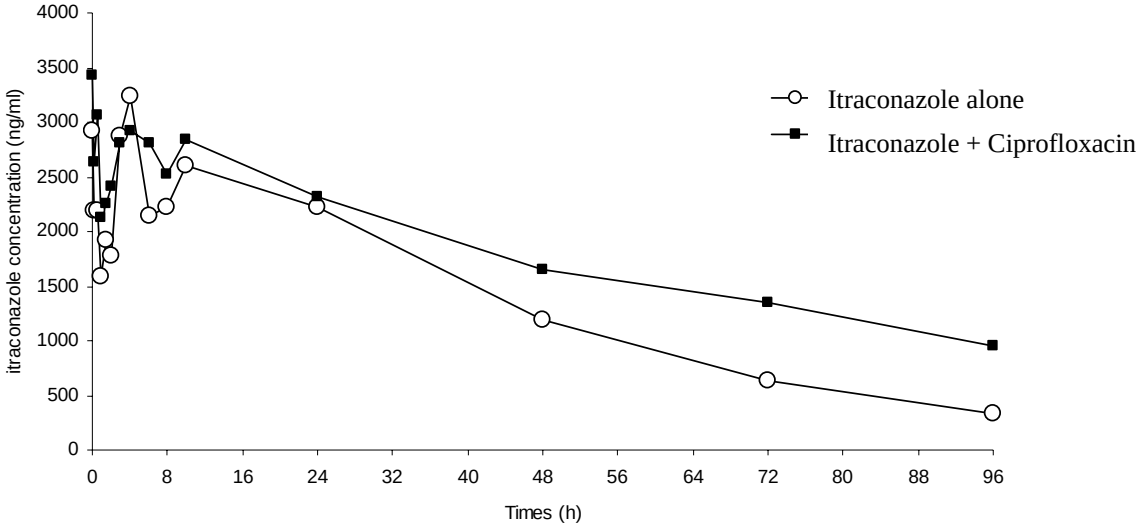

Figure 39. Plasma itraconazole concentration-time curve of subject No. 9

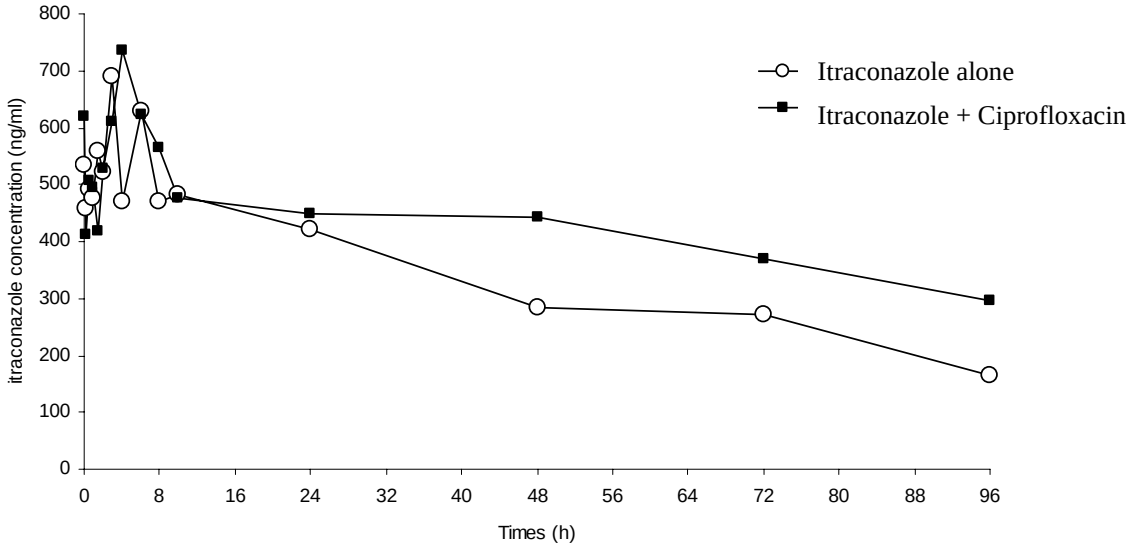

Figure 40. Plasma itraconazole concentration-time curve of subject No. 10

APPENDIX F

Table 7 Plasma ciprofloxacin concentration of each volunteer during ciprofloxacin alone

Subject	**Plasma ciprofloxacin concentration (ng/mL) at each time of blood drown (h)**												
No.	**0**	**0.25**	**0.5**	**1**	**1.5**	**2**	**3**	**4**	**6**	**8**	**10**	**24**	**48**
1	314.45	522.90	1501.35	3739.11	2452.65	2216.10	1648.27	1243.18	798.70	644.09	487.51	102.25	<LLOQ
2	443.34	427.65	485.16	851.66	1279.44	1389.97	2685.33	2621.75	1603.16	1182.13	899.15	180.22	36.00
3	351.75	345.98	402.73	956.57	2133.64	1909.59	1197.12	776.07	488.61	425.51	154.95	105.22	105.17
4	297.40	379.14	1027.23	2281.75	2418.96	2153.59	1626.81	1247.82	838.46	596.70	546.04	145.98	76.63
5	869.68	824.06	1403.85	2836.79	2569.72	2402.56	1924.16	1607.85	1159.41	898.43	640.91	136.23	32.82
6	392.35	428.34	1888.78	5063.48	3303.13	2397.97	1635.84	1306.65	1036.04	668.76	524.16	140.97	74.89
7	424.34	458.52	1635.46	3393.87	2754.72	1989.69	1581.16	1275.05	928.71	767.15	645.23	166.92	<LLOQ
8	356.11	507.17	2173.48	3112.92	2487.53	2047.20	1527.30	1230.28	949.55	701.94	587.46	427.82	71.20
9	396.66	1309.38	3616.17	3078.91	2317.91	1953.98	1503.82	1297.64	842.12	699.70	594.98	163.58	81.10
10	326.93	308.32	773.10	2892.05	2597.25	1867.61	1344.08	1078.88	792.07	578.06	482.52	76.68	<LLOQ
Mean	417.30	551.15	1490.73	2820.71	2431.49	2032.83	1667.39	1368.52	943.68	716.25	556.29	164.59	51.92
SD	165.86	302.16	948.42	1247.31	510.27	294.81	406.15	486.45	291.11	205.12	184.84	97.97	34.23

LLOQ = 31.25 ng/mL

Table 8 Plasma ciprofloxacin concentration of each volunteer during combination with itraconazole

Subject	**Plasma ciprofloxacin concentration (ng/mL) at each time of blood drown (h)**												
No.	**0**	**0.25**	**0.5**	**1**	**1.5**	**2**	**3**	**4**	**6**	**8**	**10**	**24**	**48**
1	454.82	429.10	421.56	614.97	1627.58	2124.45	1619.43	1324.88	1070.07	675.82	578.31	164.26	108.83
2	509.88	481.73	522.30	1834.48	2093.56	2508.26	2661.73	2186.06	1643.78	1200.41	927.24	208.85	46.08
3	321.17	304.21	285.22	280.04	315.34	391.19	3405.86	2070.98	1071.16	808.49	544.74	167.97	110.94
4	388.92	1123.46	4147.85	3374.96	2687.87	2049.88	1466.58	1080.05	729.80	565.28	508.76	125.44	74.29
5	958.45	911.47	957.92	955.72	1810.38	2427.12	2443.27	2220.85	1553.32	1103.00	922.45	208.01	63.63
6	370.19	389.85	2057.69	4005.61	2978.82	2493.79	1836.38	1366.03	839.84	668.53	629.01	135.07	74.11
7	470.75	526.17	682.34	1442.01	2469.90	3363.00	1977.19	1656.22	1229.65	972.87	800.51	185.64	38.07
8	139.17	232.64	2158.22	2824.80	2367.33	1854.87	1224.47	986.81	727.78	504.58	404.01	118.10	51.06
9	605.28	871.38	1680.34	2186.42	2121.57	2295.60	2145.20	1723.45	1193.56	894.93	687.05	199.34	87.56
10	335.35	327.63	1411.11	2229.67	2685.98	2279.85	1667.79	1248.96	862.69	640.78	459.03	79.80	<LLOQ
Mean	455.40	559.76	1432.45	1974.87	2115.83	2178.80	2044.79	1586.43	1092.16	803.47	646.11	159.25	66.49
SD	216.75	301.46	1169.39	1200.94	757.16	746.52	648.10	455.50	321.02	233.67	185.16	43.43	31.54

LLOQ = 31.25 ng/mL

Table 9 Effect of itraconazole on ciprofloxacin pharmacokinetics in each of ten healthy volunteers

Subject No.	Ciprofloxacin											
	C_{max} (ng/mL)		AUC_{0-48} (ng.h/mL)		$AUC_{0-\infty}$ (ng.h/mL)		$AUMC_{0-48}$ (ng.h^2/mL)		$AUMC_{0-\infty}$ (ng.h^2/mL)		MRT_{0-48} (h)	
	A	C	A	C	A	C	A	C	A	C	A	C
1	3739.11	2124.45	17820.03	19188.72	17885.98	20881.63	129469.33	225134.13	133231.52	332728.85	7.27	11.73
2	2685.33	2661.73	25690.19	27793.40	26090.98	28309.53	240374.27	262367.47	264074.30	292922.44	9.36	9.44
3	2133.64	3405.86	11948.98	19966.62	13685.47	22083.37	146911.25	233548.20	258934.51	375538.69	12.29	11.70
4	2418.96	4147.85	18913.16	19475.55	20275.99	20727.08	192768.05	177003.06	282421.99	258159.40	10.19	9.09
5	2836.79	2443.27	21907.52	27092.80	22295.51	27864.44	183271.46	267954.21	206481.03	314350.92	8.37	9.89
6	5063.48	4005.61	21622.06	21667.76	22727.81	22915.19	194220.65	197465.06	263622.74	278339.33	8.98	9.11
7	3393.87	3363.00	20920.03	24178.98	21276.99	24637.99	187606.63	224588.41	209064.44	252154.75	8.97	9.29
8	3112.92	2824.80	25680.27	16130.23	26884.98	16964.11	324614.06	148376.52	402824.15	202021.07	12.64	9.20
9	3616.17	2295.60	21271.83	24041.23	22704.84	25245.11	209468.92	248213.28	303574.75	322551.05	9.85	10.32
10	2892.05	2685.98	15628.39	16681.81	15666.17	16780.77	111847.58	119021.83	113970.00	124716.07	7.16	7.13
Mean	3189.23	2995.82	20140.25	21621.71	20949.47	22640.92	192055.22	210367.22	243819.94	275348.26	9.51	9.69
SD	831.28	702.50	4256.29	4058.49	4220.93	3998.61	60277.32	49302.62	83710.40	71723.31	1.84	1.35
***p*-value**	0.576		0.330		0.291		0.478		0.368		0.777	

A = alone, C = combination

Table 9 Effect of itraconazole on ciprofloxacin pharmacokinetics in each of ten healthy volunteers (continue)

Subject No.	Ciprofloxacin											
	$MRT_{0-\infty}$(h)		$\lambda_z(h^{-1})$		$T_{1/2,z}$ (h)		T_{max} (h)		CL/f (L/h)		$V_{z/f}$ (L)	
	A	C	A	C	A	C	A	C	A	C	A	C
1	7.45	15.93	0.11	0.06	6.27	10.78	1.00	2.00	0.03	0.02	0.25	0.37
2	10.12	10.35	0.09	0.09	7.72	7.76	3.00	3.00	0.02	0.02	0.21	0.20
3	18.92	17.01	0.06	0.05	11.45	13.22	1.50	3.00	0.04	0.02	0.60	0.43
4	13.93	12.46	0.06	0.06	12.33	11.68	1.50	0.50	0.02	0.02	0.44	0.41
5	9.26	11.28	0.08	0.08	8.19	8.41	1.00	3.00	0.02	0.02	0.27	0.22
6	11.60	12.15	0.07	0.06	10.23	11.67	1.00	1.00	0.02	0.02	0.32	0.37
7	9.83	10.23	0.08	0.08	8.40	8.36	1.00	2.00	0.02	0.02	0.28	0.24
8	14.98	11.91	0.06	0.06	11.73	11.32	1.00	1.00	0.02	0.03	0.31	0.48
9	13.37	12.78	0.06	0.07	12.25	9.53	0.50	2.00	0.02	0.02	0.39	0.27
10	7.27	7.43	0.12	0.10	5.67	6.61	1.00	1.50	0.03	0.03	0.26	0.28
Mean	11.67	12.15	0.08	0.07	9.42	9.93	1.25	1.90	0.02	0.02	0.33	0.33
SD	3.66	2.76	0.02	0.02	2.49	2.11	0.68	0.91	0.01	0.001	0.12	0.10
***p*-value**	0.643		0.275		0.412		0.051		0.290		0.829	

A = alone, C = combination

APPENDIX G

Table 10 Plasma itraconazole concentration of each volunteer during itraconazole alone

Subject No.	Plasma itraconazole concentration (ng/mL) at each time of blood drown (h)														
	0	**0.25**	**0.5**	**1**	**1.5**	**2**	**3**	**4**	**6**	**8**	**10**	**24**	**48**	**72**	**96**
1	749.76	694.37	676.54	666.52	776.08	587.55	764.95	1072.59	1020.66	942.65	674.49	552.33	421.99	378.19	227.77
2	1671.73	1248.21	1492.16	1269.93	931.72	1168.32	1193.46	1161.81	934.95	1067.64	1411.54	900.63	1127.10	846.11	517.26
3	1798.73	1824.44	1611.37	1498.88	1655.11	1445.37	1613.73	2028.06	1944.52	1624.87	1776.53	1261.85	582.43	423.57	111.48
4	1327.32	838.70	919.52	859.45	939.58	940.43	1513.98	1959.56	1699.59	1228.22	949.53	851.98	746.42	687.77	533.29
5	1641.18	1409.49	1385.51	1237.11	1353.60	1350.45	1809.53	1917.32	1814.01	1786.35	1448.15	1354.69	827.20	621.79	441.03
6	1756.88	1782.71	1685.86	1426.67	1844.82	2049.95	2821.66	3218.17	3114.33	2140.46	2008.85	1735.63	1440.66	1100.27	997.90
7	2584.18	2578.10	2242.13	2358.55	1975.32	2127.62	2392.94	2912.95	2894.12	2297.42	2592.00	1989.76	1802.94	1256.75	899.97
8	276.73	310.52	275.37	361.18	320.64	266.30	483.82	396.51	320.63	253.89	270.25	206.04	231.55	123.32	66.89
9	2917.45	2194.04	2191.53	1592.11	1926.38	1770.70	2870.87	3235.45	2139.40	2214.82	2596.14	2218.80	1194.49	628.18	338.36
10	534.11	458.68	491.45	477.46	559.42	522.38	691.56	470.91	629.58	471.57	482.22	420.25	284.99	270.28	163.51
Mean	1525.81	1333.93	1297.14	1174.79	1228.26	1222.91	1615.65	1837.33	1651.18	1402.79	1420.97	1149.20	865.98	633.62	429.75
SD	843.58	760.89	683.90	602.11	602.81	646.41	864.04	1057.27	928.77	725.87	830.67	681.23	518.29	358.40	318.92

LLOQ = 50 ng/mL

Table 11 Plasma itraconazole concentration of each volunteer during combination with ciprofloxacin

Subject No.	Plasma itraconazole concentration (ng/mL) at each time of blood drown (h)														
	0	**0.25**	**0.5**	**1**	**1.5**	**2**	**3**	**4**	**6**	**8**	**10**	**24**	**48**	**72**	**96**
1	1374.47	1838.72	1360.59	1225.12	1270.09	1733.08	1671.51	2181.99	1814.43	1863.03	1040.50	1001.37	829.99	705.25	750.24
2	1942.07	1609.52	1621.91	2087.68	1743.87	2151.52	1998.41	2465.22	3067.32	1067.64	1565.18	1361.62	1462.39	1202.67	1320.73
3	946.64	1142.17	884.29	824.17	835.38	1084.21	1275.53	1447.26	1572.72	1293.85	1275.90	778.60	355.37	107.11	<LLOQ
4	1921.70	1339.18	1325.96	1657.52	2263.47	2836.43	2771.00	2825.54	2114.23	1522.70	1798.12	1178.49	873.16	430.54	428.25
5	2485.65	2258.36	2162.25	2129.08	2148.61	2129.07	4553.54	5473.75	4839.74	4733.78	4633.50	3556.87	2536.90	1613.79	1256.47
6	2636.06	2649.20	2040.60	2374.13	2465.75	2713.79	2669.16	2696.94	2628.39	2737.21	3920.23	2027.94	1530.76	1296.86	1274.66
7	3061.51	2313.43	2582.47	2722.71	2759.37	2642.48	4713.60	4359.70	3504.55	2861.93	2917.64	2578.50	2482.14	1575.21	1587.47
8	717.04	661.98	1086.14	869.29	885.58	801.02	959.52	1451.45	1278.23	1372.05	1304.72	1118.51	797.44	447.70	335.35
9	3422.33	2636.99	3062.80	2129.04	2248.76	2414.07	2807.15	2919.69	2805.35	2531.28	2848.10	2316.13	1646.45	1351.40	954.40
10	620.31	413.11	506.84	493.81	418.59	527.10	527.10	736.68	623.35	564.00	477.44	447.43	442.85	368.95	294.69
Mean	1912.78	1686.26	1663.38	1651.25	1703.95	1903.28	2394.65	2655.82	2424.83	2054.75	2178.13	1636.55	1295.75	909.95	821.21
SD	987.37	793.55	794.37	755.31	800.55	833.75	1410.48	1406.30	1218.00	1205.69	1347.55	960.16	775.28	556.86	535.48

LLOQ = 50 ng/mL

Table 12 Effect of ciprofloxacin on itraconazole pharmacokinetics in each of ten healthy volunteers

Subject No.	Itraconazole											
	C_{max} (ng/mL)		**AUC_{0-96} (ng.h/mL)**		**$AUC_{0-\infty}$ (ng.h/mL)**		**$AUMC_{0-96}$ (ng.h^2/mL)**		**$AUMC_{0-\infty}$ (ng.h^2/mL)**		**MRT_{0-96} (h)**	
	A	C	A	C	A	C	A	C	A	C	A	C
1	1072.59	2181.99	45811.33	89187.15	65473.03	254459.04	1745892.97	3653178.28	5330634.34	55927422.99	38.11	40.96
2	1671.73	3067.32	91967.45	138853.91	123845.63	333580.75	3922287.74	6126361.96	8947207.17	53530427.47	42.65	44.12
3	2028.06	1572.72	79409.97	47775.35	83281.57	47972.28	2319758.45	1117838.90	2825888.82	1140706.32	29.21	23.40
4	1959.56	2836.43	77237.88	92647.58	163032.50	116608.10	3186259.34	3006565.59	25225095.44	6647349.41	41.25	32.45
5	1917.32	5473.75	92663.78	256675.59	126321.96	337600.13	3340326.10	9337935.28	9140238.50	22318741.30	36.05	36.38
6	3218.17	3920.23	144634.10	176709.54	258507.32	372956.79	5767630.08	6818352.65	29693895.00	55872401.30	39.88	38.59
7	2912.95	4713.60	165490.02	219397.19	227662.70	412603.87	6502678.79	8955532.55	16766341.25	51018015.61	39.29	40.82
8	483.82	1451.45	18380.11	76174.74	20965.93	96587.43	685116.34	2744431.75	1033313.52	5946570.46	37.27	36.03
9	3235.45	3422.33	131985.33	174287.46	144907.17	251625.10	4165375.34	6722615.75	5899349.05	20413912.78	31.56	38.57
10	691.56	736.69	32007.30	40654.35	46163.16	75380.45	1235489.13	1753651.55	3820009.01	9179519.35	38.60	43.14
Mean	1919.12	2937.65	87958.73	131236.29	126016.10	229937.39	3287081.43	5023646.43	10868197.21	28199506.70	37.39	37.45
SD	986.93	1501.86	48460.66	73746.13	76391.96	135339.64	1884772.44	2953079.03	9823024.22	23203908.82	4.18	6.04
***p*-value**	0.018		0.023		0.008		0.020		0.032		0.970	

A = alone, C = combination

Table 12 Effect of ciprofloxacin on itraconazole pharmacokinetics in each of ten healthy volunteers (continue)

Subject No.	Itraconazole											
	$MRT_{0-\infty}$ (h)		λ_z (h^{-1})		$T_{1/2,z}$ (h)		T_{max} (h)		CL/f (L/h)		$V_{z/f}$ (L)	
	A	C	A	C	A	C	A	C	A	C	A	C
1	81.42	219.79	0.012	0.005	59.83	152.69	4.00	4.00	0.0031	0.0008	0.26	0.17
2	72.24	160.47	0.016	0.007	42.72	102.20	0.00	6.00	0.0016	0.0006	0.10	0.09
3	33.93	23.78	0.029	0.050	24.07	13.94	4.00	6.00	0.0024	0.0042	0.08	0.08
4	154.72	57.01	0.006	0.018	111.51	38.78	4.00	2.00	0.0012	0.0017	0.20	0.10
5	72.36	66.11	0.013	0.016	52.90	44.64	4.00	4.00	0.0016	0.0006	0.12	0.04
6	114.87	149.81	0.009	0.007	79.10	106.72	4.00	10.00	0.0008	0.0005	0.09	0.08
7	73.65	123.65	0.015	0.008	47.88	84.36	4.00	3.00	0.0009	0.0005	0.06	0.06
8	49.29	61.57	0.026	0.016	26.79	42.192	3.00	4.00	0.0095	0.0021	0.37	0.13
9	40.71	81.13	0.026	0.012	26.47	56.17	4.00	0.00	0.0014	0.0008	0.05	0.06
10	82.75	121.78	0.012	0.009	60.01	81.68	3.00	4.00	0.0043	0.0027	0.38	0.31
Mean	77.59	106.51	0.02	0.01	53.13	72.34	3.40	4.30	0.0027	0.0015	0.17	0.11
SD	35.78	59.38	0.01	0.01	27.00	41.14	1.27	2.67	0.0026	0.0012	0.12	0.08
***p*-value**	0.178		0.635		0.203		0.394		0.145		0.041	

A = alone, C = combination

APPENDIX H

Drug interactions involving CYP3A4 isoenzymes

	Drugs affected (substrates)
INHIBITORS	
Azole antifungals:	Midazolam, tacrolimus, terfenadine, triazolam
Itraconazole	Cisapride, quinidine, astemizole, methylprednisolone,
Ketoconazole	buspirone,felodipine, vincristine, atorvastatin
	Terfenadine, astemizole, cyclosporine, triazolam, alprazolam
Fluconazole	Terfenadine, triazolam
Macrolide antimicrobials:	Tacrolimus, astemizole
Erythromycin	Carbamazepine, triazolam, buspirone, terfenadine, simvastatin
Clarithromycin	Pimozide, cyclosporin, midazolam
Selective serotonin re-uptake Inhibitors (SSRIs):	Midazolam, cisapride
Fluoxetine	Diazepam, alprazolam, midazolam, terfenadine
Paroxetine	Alprazolam
Calcium channel blockers:	Tacrolimus
Verapamil	Simvastatin, carbamazepine, cyclosporine
Diltiazem	Triazolam, carbamazepine, cyclosporine, quinidine, simvastatin,midazolam, alfentanil
Nifedipine	Midazolam
Protease inhibitors	Terfenadine, astemizole, cisapride, midazolam
Grapefruit juice	Felodipine, nifedipine, nimodipine, nitrendipine, terfenadine, cyclosporin, midazolam, carbamazepine, simvastatin, verapamil, prednisolone, artemether, ethinylestradiol,

Drug interactions involving CYP3A4 isoenzymes. (continue)

	Drugs affected (substrates)
Ciprofloxacin	Tacrolimus
Cimetidine	Carbamazepine, quinidine, cyclosporine, calcium channel blockers, benzodiazepines
Propofol	Midazolam
Nafimidone, omeprazole	Carbamazepine
INDUCERS	
Rifampicin	Protease inhibitors, diazepam, triazolam, midazolam, estradiol, norgesterol, lidocaine, zopiclone, zolpidem, ondansetron
Rifabutin	Protease inhibitors, estradiol, norgesterol
Phenytoin, Phenobarbitone	Midazolam, vincristine, carbamazepine
Carbamazepine	Protease inhibitors, midazolam, itraconazole, vincristine

Adapted from Badyal, D.K. and Dadhich, A.P., 2001. Cytochrome p450 and drug interactions. Indian Journal of Pharmacology, 33, 248-259.

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