เป็นหนังสือภาษาอังกฤษ

รายงานผลการวิจัย

(ฉบับสมบูรณ์)

เรื่อง

ผลของคีโตโคนาโซลต่อเภสัชจลนศาสตร์ของ ริสเพอริโดนในอาสาสมัครชายไทยสุขภาพปกติ

(Effect of Ketoconazole on the Pharmacokinetics of Risperidone in Healthy Thai Male Volunteers)

คณะผู้วิจัย :   ผศ. นพ. วีรวัฒน์ มหัทธนตระกูล

ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์

โทร. 074-288174 E-mail : werawath@gmail.com

ภก. วีระชัย พิพัฒน์รัตนเสรี

ศูนย์วิทยาศาสตร์การแพทย์สงขลา กรมวิทยาศาสตร์การแพทย์ กระทรวงสาธารณสุข

โทร. 074-447024-8 E-mail : ta068@hotmail.com

ผศ. ดร. จุติมา บุญเลี้ยง

ภาควิชาเภสัชเคมี คณะ เภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์

โทร. 074-288865 E-mail : jutima@pharmacy.psu.ac.th

อาจารย์ สมชาย ศรีวิริยะจันทร์

ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์

โทร. 074-288392 E-mail : Somchai.sr@psu.ac.th

CONTENT

ผลของคีโตโคนาโซลต่อเภสัชจลนศาสตร์ของริสเพอริโดนในอาสาสมัครชายไทยสุขภาพปกติ

บทคัดย่อ

วัตถุประสงค์ของการวิจัยครั้งนี้ คือ เพื่อศึกษาผลของยาคีโตโคนาโซลต่อเภสัชจลนศาสตร์ของยาริสเพอริโดนขนาดรับประทานครั้งเดียว เมื่อบริหารยาร่วมกันในอาสาสมัครสุขภาพปกติ การทดลองนี้แบ่งเป็น 2 ช่วงการทดลองโดยห่างกัน 2 สัปดาห์ ในแต่ละช่วงการทดลองประกอบด้วยอาสาสมัคร 2 กลุ่มๆละ 5 คน โดยในช่วงแรก กลุ่มที่ 1 อาสาสมัครรับประทานยาริสเพอริโดน 2 มก. ครั้งเดียว ส่วนกลุ่มที่ 2 อาสาสมัครรับประทานยาคีโตโคนาโซลขนาด 200 มก.วันละหนึ่งครั้งเป็นเวลา 3 วันติดต่อกันก่อนรับประทานยาริสเพอริโดน เก็บตัวอย่างเลือดครั้งละ 5 มล. ที่เวลา 0 (ก่อนรับประทานยา), 15, 30, 45 นาที, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72 และ 96 ชั่วโมง หลังรับประทานยาริสเพอริโดน ส่วนในการทดลองช่วงที่ 2 อาสาสมัครได้รับยาโดยสลับกลุ่มกับในช่วงที่ 1 และเก็บตัวอย่างเลือดเช่นเดียวกับช่วงที่ 1 วิเคราะห์ปริมาณยาริสเพอริโดน และ 9-hydroxyrisperidone ในพลาสมาโดยเทคนิค Liquid Chromatography Tandem Mass Spectrometry ซึ่งดัดแปลงจากวิธีวิเคราะห์ของ Remmeries และคณะ การสกัดยาจากพลาสมา ใช้วิธี liquid-liquid extraction และใช้ carbamazepine เป็น internal standard ความเข้มข้นของยาริสเพอริโดนที่วิเคราะห์จากพลาสมาจะถูกนำไปใช้คำนวณหาค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ โดยใช้ noncompartment model และใช้โปรแกรม WinNonlin software (Scientific Consulting, Inc., 1995) ผลการทดลองพบว่า หลังจากได้รับยาคีโตโคนาโซล อัตราการกำจัดยาลดลง 39.82% (0.0249 ± 0.0176 จาก 0.04138 ± 0.0269 ล./ชม./กก.; $P < 0.05$) ทำให้ค่าครึ่งชีวิตของการกำจัดยาริสเพอริโดนเพิ่มขึ้น 32.8% (6.46 ± 5.32 จาก 4.87 ± 3.23 ชม.; $P < 0.05$), พื้นที่ใต้กราฟระหว่างความเข้มข้นของยาริสเพอริโดนในพลาสมาตั้งแต่เวลา 0-48 และ 0-96 ชั่วโมง เพิ่มขึ้น 53.7% (109.47 ± 60.85 จาก 71.21 ± 45.56 นก./ล./ชม.; $P < 0.05$) และ 55.37% (113.71 ± 63.31 จาก 73.19 ± 46.35 นก./ล./ชม; $P < 0.05$) ตามลำดับ ส่วนความเข้มข้นสูงสุดของยาริสเพอริโดนและช่วงเวลาที่ยามีความเข้มข้นสูงสุดในพลาสมาไม่แตกต่างกัน สำหรับความเข้มข้นสูงสุด และช่วงเวลาที่มีความเข้มข้นสูงสุดของ 9-hydroxyrisperidone (9OHRIS) ในพลาสมา รวมทั้งค่าครึ่งชีวิตของการกำจัดยาไม่แตกต่างกัน ในขณะที่อัตราการกำจัด 9OHRIS เพิ่มขึ้น 117.32% (0.0115 ± 0.0057 จาก 0.0053 ± 0.0027 ล./ชม./กก.; $P < 0.05$) ซึ่งทำให้พื้นที่ใต้กราฟระหว่างความเข้มข้นของ 9OHRIS ในพลาสมาตั้งแต่เวลา 0–48 และ 0-96 ชั่วโมงลดลง 50.05% (221.65 ± 123.67 จาก 423.74 ± 163.46 นก./ล./ชม.; $P < 0.05$) และ 49.95% (220.76 ± 127.11 จาก 441.08 ± 171.63 นก./ล./ชม.; $P < 0.05$) ตามลำดับ โดยสรุป ยาคีโตโคนาโซลทำให้ยาริสเพอริโดนถูกกำจัดออกจากร่างกายลดลงอย่างมีนัยสำคัญทางสถิติ เนื่องจากยาคีโตโคนาโซลมีผลยับยั้งการทำงานของเอนไซม์ CYP3A4 ทำให้ยาริสเพอริโดนถูกแปรรูปลดลง ดังนั้นในกรณีที่มีการใช้ยาทั้ง 2 ชนิดนี้ร่วมกัน จึงควรพิจารณาปรับลดขนาดยาริสเพอริโดน และควรติดตามผลการรักษาอย่างใกล้ชิด

Effect of Ketoconazole on the Pharmacokinetics of Risperidone in Healthy Thai Male Volunteers

ABSTRACT

The objective of this study is to investigate the effect of ketoconazole on the pharmacokinetics of a single oral dose of risperidone in healthy volunteers. In the present study, an open-lebel, randomized, two phases crossover design with a 2 week washout period was performed. In phases 1, each subject in group 1 ingested a single dose of 2 mg of risperidone and in group 2, each subject ingested the same dose of risperidone after orally pretreatment with 200 mg of ketoconazole once daily for 3 days. Blood samples were collected at specific times after ingestion of risperidone (i.e. 0, 15, 30, 45 min and 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, and 96 hr). In phase 2, the volunteers received the medication by crossover method. Plasma samples were collected as same as in phase 1. Plasma concentration of risperidone and 9-hydroxyrisperridone were determined by the modified method of Remmerie et al using LC-MS/MS. Liquid-liquid extraction were used for the extraction and carbamazepine was used as internal standard. The pharmacokinetic parameters were determined from the plasma concentration of risperidone and 9-hydroxyrisperidone using the WinNonlin software (Scientific Consulting, Inc., 1995). The validation method was reliable. The results had shown that after pretreatment with ketoconazole, the clearance of risperidone is significantly decreased by 39.82% (0.0249 ± 0.0176 vs 0.04138 ± 0.0269 l/hr/kg; $P < 0.05$). The half-life of risperidone was significantly increased by 32.8% (6.46 ± 5.32 vs 4.87 ± 3.23 hr; $P < 0.05$). The AUC_{0-48} and AUC_{0-96} of risperidone were also significantly increased by 53.7% (109.47 ± 60.85 vs 71.21 ± 45.56 ng/l/hr; $P < 0.05$) and 55.37% (113.71 ± 63.31 ng/l/hr; $P < 0.05$), respectively. However, the C_{max} and T_{max} of risperidone were not significantly changed. The C_{max}, T_{max} and $T_{1/2}$ of 9-hydroxyrisperidone (9OHRIS) were also not significantly changed. While the Cl/f of 9OHRIS was significantly increased by 117.32% (0.0115 ± 0.0057 vs 0.0053 ± 0.0027 l/hr/kg; $P < 0.05$). These changes led to corresponding decrease in the AUC_{0-48} and AUC_{0-96} of 9OHRIS which were significantly decreased by 50.05% (211.65 ± 123.67 vs 423.74 ± 163.46 ng/l/h; $P < 0.05$), and 49.95% (220.76 ± 127.11 vs 441.08 ± 171.63 ng/l/hr; $P < 0.05$), respectively. In conclusion, ketoconazole significantly inhibits the metabolism of risperidone, due to inhibition of CYP 3A4. Therefore, dosage of risperidone should be decreased when it is coadministered with ketoconazole and the patient should be closely monitored.

INTRODUCTION

Schizophrenia is one of the most disabling and emotionally devastating illnesses known to man. Schizophrenia is a complex disorder comprised of different cluster of signs and symptoms. The severity of the symptoms and long-lasting, chronic pattern of schizophrenia, often cause a high degree of disability. The mortality rate of schizophrenia is estimated to be twice that of the general population. Approximately 10% of the mortality is secondary to suicide. Young male schizophrenics are most likely to complete suicide attempts, especially early in their illness (Travers D *et al.*, 2008). Schizophrenia is a very expansive illness because it usually affects people when they are young and it has a chronic course throughout the rest of their lives. Most people with schizophrenia experience multiple hospitalizations and account for about 40% of hospital beds occupied (Drake *et al.*, 2000). The features characteristic of schizophrenia is classified as positive, negative and disorganization symptoms (Andreasen *et al.*, 1990). The symptoms that are most commonly associated with the disease are called positive symptoms, that denote the presence of grossly abnormal behavior. These include thought disorder, delusions, and hallucinations. Thought disorder is the diminished ability to think clearly and logically. Often it is manifested by disconnected and nonsensical language that renders the person with schizophrenia incapable of participating in conversation, contributing to his alienation from his family, friends, and society. Delusions are common among individuals with schizophrenia. An affected person may believe that he is being conspired against (called "paranoid delusion"). "Broadcasting" describes a type of delusion in which the individual with this illness believes that his thoughts can be heard by others. Hallucinations can be heard, seen, or even felt; most often they take the form of voices heard only by the afflicted person. Such voices may describe the person's actions, warn him of danger or tell him what to do. At times the individual may hear several voices carrying on a conversation. Less obvious than the "positive symptoms" but equally serious are the deficit or negative symptoms that represent the absence of normal behavior. These include flat or blunted affect (i.e. lack of emotional expression), apathy, and social withdrawal). Negative symptoms include social and emotion withdrawal, apathy, avolition, attention deficit, alogia, blunted affect, poor eye contact and poor insight and adjustment. Disorganization consists of incoherence, loose association, inappropriate affect and poverty of thought content.

Antipsychotic medications have been available since the mid-1950s. They effectively alleviate the positive symptoms of schizophrenia. While these drugs have greatly improved the lives of many patients, they do not cure schizophrenia. Everyone responds differently to antipsychotic medication. Sometimes several different drugs must be tried before the right one is found. People with schizophrenia should work in partnership with their doctors to find the medications that control their symptoms best with the fewest side effects. The older antipsychotic medications include chlorpromazine, haloperidol, perphenazine, and fluphenazine. The atypical antipsychotic drugs such as risperidone, olanzapine, quetiapine, sertindole, and ziprasidone are effective and rarely produce extrapyramidal symptoms who are taking

atypical antipsychotics should not drive until they adjust to their new medication. If people with schizophrenia become depressed, it may be necessary to add an antidepressant to their drug regimen. A large clinical trial funded by the National Institute of Mental Health (NIMH), known as CATIE (Clinical Antipsychotic Trials of Intervention Effectiveness), compared the effectiveness and side effects of five antipsychotic medications both new and older antipsychotics that are used to treat people with schizophrenia.

Medications and other treatments for schizophrenia, when used regularly and as prescribed, can help reduce and control the distressing symptoms of the illness. However, some people are not greatly helped by available treatments or may prematurely discontinue treatment because of unpleasant side effects or other reasons. Even when treatment is effective, persisting consequences of the illness lost opportunities, stigma, residual symptoms, and medication side effects may be very troubling. In recent years, the possibility that additional neurotransmitters, acting in concert with dopamine (DA), contribute to the etiology of schizophrenia and action of antipsychotics drugs has received more attention than DA (Bersani *et al.*, 2006). The serotonin (5-HT) antagonists such as methysergide, cyproheptadine and ritanserin are not psychotomimetic and may possibly have beneficial effects on specific aspects of psychosis. Furthermore, antipsychotics such as clozapine, risperidone, planzapine and ziprasidone antagonize at multiple 5-HT receptors and have some advantages over selective DA receptor antagonists. There is now considerable evidence from studies of schizophrenia and other forms of psychoses, that the atypical antipsychotic drugs such as clozapine, risperidone, olanzapine and ziprasidone are more effective with fewer extrapyramidal symptoms (EPS). These drugs have higher potencies as $5-HT_{2A}$ antagonists than as D_2 antagonists or that almost any other receptor. At clinically effective dose, they all produce fewer EPS than typical neuroleptic drug. However, marked EPS can occur with risperidone if the dose is increased. Risperidone is a new medication for treating schizophrenia and psychotic disorders. It helps manage schizophrenia's "positive symptoms" such as visual and auditory hallucinations, delusions, and thought disturbances. Risperidone may also help in treating what so-called "negative symptoms" such as social withdrawal, apathy, lack of motivation, and inability to experience pleasure. Side effects are usually relatively minor, and blood monitoring is not necessary. Risperidone is the first new front-line treatment option in twenty years. Conventional antipsychotics such as Haldol, Stelazine, and Prolixin have been used to treated positive symptoms in many patients for several years. However, they do not satisfactorily affect the negative symptoms, and they often cause uncomfortable or intolerable side effects.

Risperidone is an atypical antipsychotic agent chemically classified as a benzisoxazole derivative. It is a selective monoaminergic antagonist with high affinity for serotonin type II ($5-HT_{2A}$) and dopamine-D_2 receptors (Nyberg *et al.*, 1993). Clinical trails in psychotic patients have shown that risperidone is effective in the treatment of the positive, negative and affective symptoms of schizophrenia (Marder *et al.*, 1997). Furthermore, risperidone therapy is associated with reduced extrapyramidal symptoms. Recently, the U.S. Food and Drug Administration (FDA)

approved the use of risperidone for the treatment of residual schizophrenia, and a large prospective clinical study comparing risperidone with haloperidol has demonstrated that patients treated with risperidone have a lower risk of relapse than those treated with haloperidol. Due to the favorable clinical effects of risperidone, a substantial increase in its use has occurred during the last few years. Risperidone is rapidly and very well absorbed after administration orally; less than 1% is excreted unchanged in the faces (Heykants et al., 1994). The principal metabolite is 9-hydroxyrisperidone. The major metabolic pathway is by CYP2D6 and CYP3A4 and the minor pathway is by N-dealkylation (Mannens et al., 1993). Hydroxylation of risperidone is subject to the same genetic CYP 2D6 related polymorphism as for debrisoquine and dextromethorphan. In poor metabolizers, the half life of risperidone was 20 hours compared with about 3 hours in extensive metabolizers (Huang et al., 1993). Plasma concentrations of risperidone, 9-hydroxyrisperidone and risperidone plus 9-hydroxyrisperidone are dose proportional over the dosing range of 1 to 16 mg daily (0.5 to 8 mg twice a day). Risperidone exhibits linear elimination kinetics. Steady state is reached within 1 day for risperidone and within 5 days for the active fraction (Leysen et al., 1988).

Azole compounds are used extensively for treatment of cutaneous and invasive fungal infections. Ketoconazole is the current drug of choice for treating systemic fungal infections such as candidiasis, blastomycosis, histoplasmosis as well as candida vulvovaginitis. This drug does not appear to have any useful antibacterial or antiparasitic activity, with the possible exception of antiprotozoal effects against Leishmania major (Chambers, 2001). Ketoconazole, a potent CYP3A4 inhibitor, is an oral antifungal agent of the imidazole class, which contains two nitrogen atoms in the five-membered azole ring (Cleary, et al., 1992). The primary mechanism of action of ketoconazole and azoles, in general, is the inhibition of sterol 14-α-dimethylase, a microsomal cytochrome P450-dependent enzyme system (Fabris, et al., 1993). Ketoconazole is dibasic compound [pK_a (1) = 6.51; pK_a (2) = 2.94] and almost insoluble in water except at a pH lower than 3, (Daneshmend, 1990). Therefore, any conditions that lower the acidity or increase the pH of stomach will decrease the absorption and hence reduce the bioavailability of ketoconazole.

Following the oral administration of single 200 mg dose of ketoconazole to eight healthy volunteers after a standard breakfast, peak serum concentrations of 3.63 ± 1.70 mg/l occurred in 2.62 ± 0.52 hr (Daneshmend, 1986). The mean half-life was reported to be 1.46 ± 0.39 h (Daneshmend, 1986). Daneshmend et al., 1984) performed a study where six healthy males were given single 200 mg dose of ketoconazole after a standard breakfast. For the 200 mg dose, mean peak concentrations of 3.60 ± 1.65 mg/l occurred in about 2 hr. The average half-life was 2.03 ± 0.42 hr. Lelawongs et al. (1988) studied the effects of food on the bioavailability of ketoconazole tablet. This study found a significant difference in the C_{max} (3.01 vs 4.37 µg/ml) and AUC_{0-24} (15.25 vs. 20.47 µg.h/ml) between the fasting group and high carbohydrate meal.

As ketoconazole is one of azole compounds, a number of side effects are associated with ketoconazole as a result of inhibition of these mammalian enzymes (Venkatakrishnan, 2000). Ketoconazole can inhibit CYP3A4, the major CYP isoform of the liver (Suzuki, 2000). The inhibitions of CYP3A4 results in drug-drug interactions involving ketoconazole can decrease the rate of clearance of many drugs (Tsunoda, 1999).

Since risperidone is metabolized by CYP3A4 (Lim *et al.*, 2009) and this enzyme is also involved in the metabolism of several other drugs (such as alprazolam, atorvastatin, carbamazepine, cyclosporine, dexamethasone, diltiazem, etc.) so pharmacokinetic interactions due to competitive inhibition of the enzyme may occur. *In vitro* studies showed that these drugs may also be metabolized by other CYP isozymes, including 1A1, 1A2 and 2C9, but they are only weak inhibitors of risperidone metabnolism.

Due to the increase in prescribing ketoconazole, the possibility of ketoconazole and risperidone coadministration tends to have a chance to occur in clinical practice, and may lead to ketoconazole-risperidone drug interaction. To our knowledge, there is no report on the possible interaction between ketoconazole and risperidone, and the possible role of cytochrome P450 enzymes in the metabolism of risperidone. Therefore, the purpose of this investigation is to study the effect of ketoconazole on the pharmacokinetics of a single oral dose of risperidone in healthy Thai male volunteers, and the present study may be used as a guidance and useful for decision making in case of coadministration of risperidone and ketoconazole in clinical practice.

I. RISPERIDONE

1. Physicochemical properties

Risperidone[3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl] 6,7,8,9-tetrahydro-2-methyl-4*H*-pyrido[1,2-a]pyrimidin-4-one (Figure 1), is a benzisoxazol derivative. Its molecular formula is $C_{23}H_{27}FN_4O_2$ and its molecular weight is 410.49. Risperidone is a white to slightly beige powder. It is practically insoluble in water, freely soluble in methylene chloride, and soluble in methanol and 0.1 N HCl.
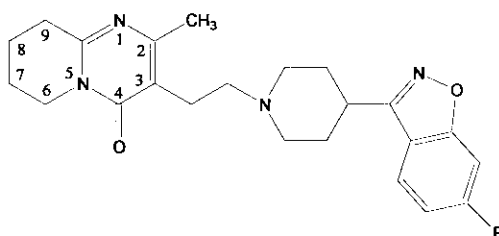

Figure1 Structural formula of risperidone (Lostia *et al.*, 2009).

2. Mechanism of action.

Mechanism of action of antipsychotics in schizophrenia treatment is still unclear. Risperidone was selected for treatment in patients with schizophrenia because of its potent antagonistic effects to serotonin $5\text{-}HT_2$ and dopamine D_2 receptor. There are many published papers which have been shown that risperidone is serotonin $5\text{-}HT_2$ and dopamine D_2 antagonist (Arman S., *et al.*, 2008). Kapur, *et al.*, 2002 represented occupancy of clozapine, risperidone, and olanzapine to serotonin $5\text{-}HT_2$ and dopamine D_2 receptor by using positron emission tomography imaging in forty-four patient with schizophrenia. The result showed that all three drugs showed greater $5\text{-}HT_2$ than D_2 occupancy at all doses (Bruins *et al.*, 2008).

3. Pharmacokinetic Properties

Absorption

The pharmacokinetics of risperidone has been investigated by manufacturer in healthy male volunteers. Risperidone is well absorbed from gastrointestinal tract produce peak plasma concentration within 1 hour in extensive metabolizers and within 2 hours in poor metabolizers (Hardy *et al.*, 2006). Oral absorption of the drug is not affected by food (Cope *et al.*, 2008). The absolute oral bioavailability of risperidone in extensive metabolizers is approximately 66% and 45% in poor metabolizers. In consideration of active moiety, the absolute bioavailability is 100%. Plasma concentration of risperidone and 9-hydroxyrisperidone are dose proportional over the dosing range of 1 to 16 mg daily (Gutierrez *et al* 1997). A study which compared oral bioavailability of a 1 mg tablet with a 1 mg/ml oral solution demonstrated bioequivalence of these two drug products with relative oral bioavailability of 95% (Eichenbaum *et al.*, 2006).

Distribution

Risperidone are rapidly distributed in body with apparent volume of distribution at steady state approximately 1.3 l/kg. Risperidone and 9-hydroxyrisperidone bind to albumin and alpha 1-acid glycoprotein approximately 90% and 77%, respectively (Mannens *et al.*, 1994).

Metabolism

Risperidone is metabolized to major therapeutically active metabolite, 9-hydroxyrisperidone (Figure 2), which is equipotent pharmacological effect to the parent compound. Risperidone is mainly metabolised via hydroxylation pathway by microsomal enzyme, cytochrom P450 subtype 2D6 and 3A4. The minor metabolic pathway is oxidative *N*-dealkylation, which result in two acidic non-pharmacologically active. Hydroxylation of risperidone by cytochrom P450 subtype 2D6 subjects to genetic polymorphism (Mannens *et al.*, 1993). Therefore, some individuals, approximately 90% of the Caucasian and 99% of the Oriental population, are extensive metabolizers, while others are intermediate or poor metabolizers. In extensive metabolizers, peak plasma concentration of 9-hydroxyrisperidone will achieves within 3 hours (Owen, 2007).
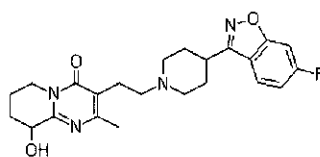
Figure 2 Structural formula of 9-hydroxyrisperidone (Janssen *et al.*, 1988)

Excretion

Within 1 week after oral administration, 70% of risperidone is excreted in urine and 14% is excreted in feces. Apparent half-life of risperidone is approximately 3 hours in extensive metabolizers and 16 hours in poor metabolizers. 9-hydroxyrisperidone has longer half-life than risperidone, i.e. approximately 20 hours in extensive metabolizers and 27 hours in poor metabolizers (Sproule *et al.*, 1997). Pharmacokinetic parameters of active moiety are not significantly difference from parent and metabolite. Overall half-life is approximately 20 hours (Heykants *et al.*, 1994).

4. Indication and Dosage

Risperidone is indicated for the treatment of schizophrenia. Risperidone is initially administered by 1 mg twice a day dosing (BID) in first day of treatment. Dosage may increase in increment of 1 mg BID in second and third day, as tolerated, to target dose of 3 mg BID (Aman *et al.*, 2007). Three published papers investigated the multicenter trials of effective dose with minimal side effects in American and Canadian schizophrenia patients indicate that risperidone is effective against positive and negative symptoms in dose range of 2-6 mg daily. The optimal therapeutic dose of risperidone is 6 mg/day (Williams, 2001) which has therapeutic effectiveness in both safety and efficacy. Study on 10 chronic schizophrenia Thai patients for 6 months indicate the mean risperidone dose of 5.56 mg/day (modal risperidone dose 4 mg/day) which correlate to the previous study resulting of 2-6 mg/day risperidone (Yoshimura *et al.*, 2008) is the optimal dose range for most patients (Williams, 2001).

For elderly and patient with renal function impairment, the renal clearance of active moiety is reduced approximately 30% (Snoeck *et al.*, 1995) in the elderly and about 50% in renal disease patient. In addition, the half-life of the active moiety is prolonged, therefore dose reduction and caution dose titration is advised for these patients. The initial dose should be start with dose of 0.5 mg BID and increase in increment of 0.5 mg BID to a total dose of 1 or 2 mg.(Horrigan and Barnhill 1997).

5. Precausions

Orthostatic Hypotension

Risperidone may induce orthostatic hypotension associated with dizziness, tachycardia, and in some patients, syncope, especially during the initial dose-titration period, probably reflecting its alpha-adrenergic antagonistic properties. A dose reduction should be considered if hypotension occurs. Risperidone should be used with particular precaution in patients with known cardiovascular disease (history of myocardial infarction or ischemia, heart failure, or conduction abnormalities), cerebrovascular disease, and conditions which would predispose patients to

10

hypotension, e.g., dehydration and hypovolemia. Clinically significant hypotension has been observed with concomitant use of risperidone and antihypertensive medication (Nourian *et al.*, 2008).

Seizure

During premarketing testing, seizures occurred in 0.3% of risperidone-treated patients, two in association with hyponatremia (Whitten and Ruehter, 1997). Risperidone should be used cautiously in patients with a history of seizures (Schneider and Lizer, 2008).

Dysphagia

Esophageal dysmotility and aspiration have been associated with antipsychotic drug use. Aspiration pneumonia is a common cause of morbidity and mortality in patients with advanced Alzheimer's dementia. Risperidone and other antipsychotic drugs should be used cautiously in patients at risk for aspiration pneumonia (Duggal and Mendhekar,2008).

Hyperprolactinemia

As with other drugs that antagonize dopamine D2 receptors, risperidone elevates prolactin levels and the elevation persists during chronic administration. Tissue culture experiments indicate that approximately one-third of human breast cancers are prolactin dependent in vitro, a factor of potential importance if the prescription of risperidone is contemplated in a breast cancer patient. Although disturbances such as galactorrhea, amenorrhea, gynecomastia, and impotence have been reported with prolactin-elevating compounds, the clinical significance of elevated serum prolactin levels is unknown for most patients. As is common with compounds which increase prolactin release, an increase in pituitary gland, mammary gland, and pancreatic islet cell hyperplasia and neoplasia was observed in the risperidone carcinogenicity studies conducted in mice and rats. However, neither clinical studies nor epidemiologic studies conducted to date have shown an association between chronic administration of this class of drugs and tumorigenesis in humans; the available evidence is considered too limited to be conclusive at this time (Bostwick *et al.*, 2009).

Potential for Cognitive and Motor Impairment

Somnolence was a commonly reported adverse event associated with risperidone treatment, especially when ascertained by direct questioning of patients. This adverse event is dose-related, and in a study utilizing a checklist to detect adverse events, 41% of the high-dose patients (risperidone 16 mg/day) reported somnolence compared to 16% of placebo patients. Direct questioning is more sensitive for detecting adverse events than spontaneous reporting, by which 8% of risperidone 16 mg/day patients and 1% of placebo patients reported somnolence as an adverse event. Since risperidone has the potential to impair judgment, thinking, or motor skills, patients should be cautioned about operating hazardous machinery, including automobiles, until they are reasonably certain that risperidone therapy does not affect them adversely (Allain *et al.*, 2003).

Priapism

Rare cases of priapism have been reported. While the relationship of the events to risperidone use has not been established, other drugs with alpha-adrenergic blocking effects have been reported to induce priapism, and it is possible that

risperidone may share this capacity. Severe priapism may require surgical intervention (Rosenberg *et al.*, 2009).

Thrombotic Thrombocytopenic Purpura (TTP)

A single case of TTP was reported in a 28 year-old female patient receiving risperidone in a large, open premarketing experience (approximately 1300 patients). She experienced jaundice, fever, and bruising, but eventually recovered after receiving plasmapheresis. The relationship to risperidone therapy is unknown.

Antiemetic Effect

Risperidone has an antiemetic effect in animals; this effect may also occur in humans, and may mask signs and symptoms of overdosage with certain drugs or of conditions such as intestinal obstruction, Reye's syndrome, and brain tumor (Auclair *et al.*, 2008).

Body Temperature Regulation

Disruption of body temperature regulation has been attributed to antipsychotic agents. Both hyperthermia and hypothermia have been reported in association with oral risperidone use. Caution is advised when prescribing for patients who will be exposed to temperature extremes (Razaq and Samma, 2004).

Suicide

The possibility of a suicide attempt is inherent in schizophrenia, and close supervision of high-risk patients should accompany drug therapy. Prescriptions for risperidone should be written for the smallest quantity of tablets, consistent with good patient management, in order to reduce the risk of overdose (Isbister and Whyte, 2002).

6. Use in Patients with Concomitant Illness

Clinical experience with risperidone in patients with certain concomitant systemic illnesses is limited. Patients with Parkinson's Disease or Dementia with Lewy Bodies who receive antipsychotics, including risperidone, may be at increased risk of Neuroleptic Malignant Syndrome as well as having an increased sensitivity to antipsychotic medications. Manifestation of this increased sensitivity can include confusion, obtundation, postural instability with frequent falls, in addition to extrapyramidal symptoms (Lane *et al.*, 1998).

Caution is advisable in using risperidone in patients with diseases or conditions that could affect metabolism or hemodynamic responses. Risperidone has not been evaluated or used to any appreciable extent in patients with a recent history of myocardial infarction or unstable heart disease. Patients with these diagnoses were excluded from clinical studies during the product's premarket testing. Increased plasma concentrations of risperidone and 9-hydroxyrisperidone occur in patients with severe renal impairment (creatinine clearance <30 mL/min/1.73 m^2), and an increase in the free fraction of risperidone is seen in patients with severe hepatic impairment. A lower starting dose should be used in such patients (Snoeck *et al.*, 1995).

7. Contraindication

Risperidone is contraindicated in patients with a known history of hypersensitivity to the product.

8. Drug interactions

The interactions of risperidone and other drugs have not been systematically evaluated. Given the primary CNS effects of risperidone, caution should be used when risperidone is taken in combination with other centrally acting drugs and alcohol. Because of its potential for inducing hypotension, risperidone may enhance the hypotensive effects of other therapeutic agents with this potential.

Risperidone may antagonize the effects of levodopa and dopamine agonists. Amytriptyline does not affect the pharmacokinetics of risperidone or the active antipsychotic fraction. Cimetidine and ranitidine increased the bioavailability of risperidone, but only marginally increased the plasma concentration of the active antipsychotic fraction. Chronic administration of clozapine with risperidone may decrease the clearance of risperidone.

Carbamazepine and Other Enzyme Inducers.

In a drug interaction study in schizophrenic patients, 11 subjects received risperidone titrated to 6 mg/day for 3 weeks, followed by concurrent administration of carbamazepine for an additional 3 weeks. During co-administration, the plasma concentrations of risperidone and its pharmacologically active metabolite, 9-hydroxyrisperidone, were decreased by about 50%. Plasma concentrations of carbamazepine did not appear to be affected. The dose of risperidone may need to be titrated accordingly for patients receiving carbamazepine, particularly during initiation or discontinuation of carbamazepine therapy. Co-administration of other known enzyme inducers (e.g., phenytoin, rifampin, and phenobarbital) with risperidone may cause similar decreases in the combined plasma concentrations of risperidone and 9-hydroxyrisperidone, which could lead to decreased efficacy of risperidone treatment (Sproule et al., 1997).

Fluoxetine and Paroxetine

Fluoxetine (20 mg QD) and paroxetine (20 mg QD) have been shown to increase the plasma concentration of risperidone 2.5-2.8 fold and 3-9 fold respectively. Fluoxetine did not affect the plasma concentration of 9-hydroxyrisperidone (Dhir and Kulkarni, 2008). Paroxetine lowered the concentration of 9-hydroxyrisperidone an average of 13%. When either concomitant fluoxetine or paroxetine is initiated or discontinued, the physician should re-evaluate the dosing of risperidone. The effects of discontinuation of concomitant fluoxetine or paroxetine therapy on the pharmacokinetics of risperidone and 9-hydroxyrisperidone have not been studied (Saito et al., 2005).

Lithium

Repeated oral doses of risperidone (3 mg BID) did not affect the exposure (AUC) or peak plasma concentrations (C_{max}) of lithium (n = 13) (Demling et al., 2006).

Valproate

Repeated oral doses of risperidone (4 mg QD) did not affect the pre-dose or average plasma concentrations and exposure (AUC) of valproate (1000 mg/day in three divided doses) compared to placebo (n = 21). However, there was a 20% increase in valproate peak plasma concentration (Cmax) after concomitant administration of risperidone (Dervaux and Levasseur, 2008).

Digoxin

Risperidone (0.25 mg BID) did not show a clinically relevant effect on the pharmacokinetics of digoxin.

Drugs that Inhibit CYP 2D6 and Other CYP Isozymes.

Risperidone is metabolized to 9-hydroxyrisperidone by CYP 2D6, an enzyme that is polymorphic in the population and that can be inhibited by a variety of psychotropic and other drugs. Drug interactions that reduce the metabolism of risperidone to 9-hydroxyrisperidone would increase the plasma concentrations of risperidone and lower the concentrations of 9-hydroxyrisperidone. Analysis of clinical studies involving a modest number of poor metabolizers does not suggest that poor and extensive metabolizers have different rates of adverse effects. No comparison of effectiveness in the two groups has been made (DeVane and Nemeroff, 2001).

In vitro studies showed that drugs metabolized by other CYP isozymes, including 1A1, 1A2, 2C9, 2C19, and 3A4, are only weak inhibitors of risperidone metabolism. There were no significant interactions between risperidone and erythromycin (Fisman *et al.*, 1996).

Drugs Metabolized by CYP 2D6

In vitro studies indicate that risperidone is a relatively weak inhibitor of CYP 2D6. Therefore, risperidone is not expected to substantially inhibit the clearance of drugs that are metabolized by this enzymatic pathway. In drug interaction studies, risperidone did not significantly affect the pharmacokinetics of donepezil and galantamine, which are metabolized by CYP 2D6 (Urichuk *et al.,* 2008*).*

9. Adverse drug reactions

The following findings are based on the short-term, placebo-controlled, North American, premarketing trials for schizophrenia and acute bipolar mania, and are followed by a description of adverse events and other safety measures in short-term, placebo-controlled trials in pediatric patients treated for irritability associated with autistic disorder. In patients with Bipolar I Disorder, treatment-emergent adverse events are presented separately for risperidone as monotherapy and as adjunctive therapy to mood stabilizers. Certain portions of the discussion below relating to objective or numeric safety parameters, namely dose-dependent adverse events, vital sign changes, weight gain, laboratory changes, and ECG changes are derived from studies in patients with schizophrenia. However, this information is also generally applicable to bipolar mania and pediatric patients with autistic disorder

Table 1 Adverse events of risperidone compared with placebo in schizophrenic patients (Li, 2009).

Adverse Event	Risperidone	Placebo
EPS	2.10%	0%
Dizziness	0.70%	0%
Hyperkinesia	0.60%	0%
Somnolence	0.50%	0%
Nausea	0.30%	0%

10. Associated With Discontinuation of Treatment

Schizophrenia

Approximately 9% (244/2607) of risperidone treated patients in Phase 2 and 3 studies treatment due to an adverse event, compared with about 7% on placebo and 10% on active control drugs. The more common events ($\geq$0.3%) associated with discontinuation and considered to be possibly or probably drug-related included suicide attempt and extrapyramidal symptoms.

Suicide attempt was associated with discontinuation in 1.2% of risperidone treated patients compared to 0.6% of placebo patients, but, given the almost 40-fold greater exposure time in risperidone compared with placebo groups, it is unlikely that suicide attempt is a risperidone related adverse event. Extrapyramidal symptoms was 0% in placebo patients, but 3.8% in active-control patients treated with rispeeridone in the Phase 2 and 3 trials (Janssen *et al.*, 1988).

Bipolar Mania

In the U.S. placebo-controlled trial with risperidone as monotherapy, approximately 8% (10/134) of risperidone treated patients discontinued treatment due to an adverse event, compared with approximately 6% (7/125) of placebo-treated patients. The adverse events associated with discontinuation and considered to be possibly, probably, or very likely drug-related included paroniria, somnolence, dizziness, extrapyramidal disorder, and muscle contractions involuntary. Each of these events occurred in one risperidone-treated patient (0.7%) and in no placebo-treated patient (0%).

In the U.S. placebo-controlled trial with risperidone as adjunctive therapy to mood stabilizers, there was no overall difference in the incidence of discontinuation due adverse events (4% for risperidone vs. 4% for placebo).

11. Incidence in Controlled Trials.

Commonly Observed Adverse Events in Controlled Clinical Trials.

Schizophrenia

In two 6- to 8-week placebo-controlled trials, spontaneously-reported, treatment-emergent adverse events with an incidence of 5% or greater in at least one of the risperidone groups and at least twice that of placebo were anxiety, somnolence, extrapyramidal symptoms, dizziness, constipation, nausea, dyspepsia, rhinitis, rash, and tachycardia. Adverse events were also elicited in one of these two trials (i.e., in the fixed-dose trial comparing risperidone at doses of 2, 6, 10, and 16 mg/day with placebo) utilizing a checklist for detecting adverse events, a method that is more sensitive than spontaneous reporting. By this method, the following additional

common and drug-related adverse events occurred at an incidence of at least 5% and twice the rate of placebo: increased dream activity, increased duration of sleep, accommodation disturbances, reduced salivation, micturition disturbances, diarrhea, weight gain, menorrhagia, diminished sexual desire, erectile dysfunction, ejaculatory dysfunction, and orgastic dysfunction.

Bipolar Mania

In the U.S. placebo-controlled trial with risperidone as monotherapy, the most commonly observed adverse events associated with the use of risperidone (incidence of 5% or greater and at least twice that of placebo) were somnolence, dystonia, akathisia, dyspepsia, nausea, parkinsonism, vision abnormal, and saliva increased. In the U.S. placebo-controlled trial with risperidone as adjunctive therapy to mood stabilizers, the most commonly observed adverse events associated with the use of risperidone were somnolence, dizziness, parkinsonism, increased salivation, akathisia, abdominal pain, and urinary incontinence.

Adverse Events Occurring at an Incidence of 1% or more Among Risperidone - Treated Patients – Schizophrenia.

The table that follows enumerates adverse events that occurred at an incidence of 1% or more, and were more frequent among risperidone treated patients treated at doses of 10 mg/day than among placebo-treated patients in the pooled results of two 6- to 8-week controlled trials. Patients received risperidone doses of 2, 6, 10, or 16 mg/day in the dose comparison trial, or up to a maximum dose of 10 mg/day in the titration study. This table shows the percentage of patients in each dose group (≤ 10 mg/day or 16 mg/day) who spontaneously reported at least one episode of an event at some time during their treatment. Patients given doses of 2, 6, or 10 mg did not differ materially in these rates. Reported adverse events were classified using the World Health Organization preferred terms.The prescriber should be aware that these figures cannot be used to predict the incidence of side effects in the course of usual medical practice where patient characteristics and other factors differ from those which prevailed in this clinical trial. Similarly, the cited frequencies cannot be compared with figures obtained from other clinical investigations involving different treatments, uses, and investigators. The cited figures, however, do provide the prescribing physician with some basis for estimating the relative contribution of drug and non-drug factors to the side effect incidence rate in the population studied.

16

II. KETOCONAZOLE

Ketoconazole is a synthetic broad-spectrum antifungal agent available in white tablets, each containing 200 mg ketoconazole base for oral administration. Inactive ingredients are colloidal silicon dioxide, corn starch, lactose, magnesium stearate, microcrystalline cellulose, and povidone. Ketoconazole is cis-1- acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxyl]phenyl] piperazine (Cordoba-Diaz *et al.*, 2001).The structural formula of ketoconazole is shown in figure 2 , the first orally absorbable antifungal azole, was introduced in 1970 (Lyman and Walsh, 1992). It offered a number of significant advantages, including its synthetic broad-spectrum antifungal agent, possesses some antifungal antibacterial activity (Shuster, 1984; McGrawth and Murphy, 1991) and wide tissue distribution, but strong inhibitory effect on cyclosporin oxidase and testosterone 6 hydroxylase activity in human (Baldwin *et al.*, 1995).

Chemical and Physical Properties

Chemical structure : $C_{26}H_{28}Cl_2N_4O_4$ (Figure 2)

Molecular weight: 531.4

pKa: 2.94, 6.51

Solubility: in   alcohol: 1in 54 (w/v)

In water: almost insoluble
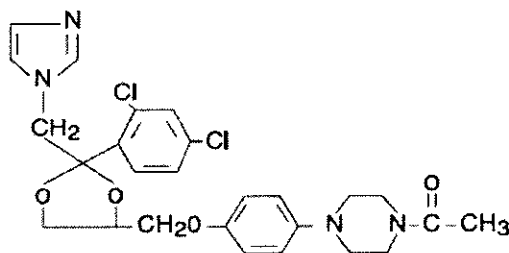
Octanol/water partition coefficient: 5400 (pH 11.8)

Figue 3 Structural Formula of Ketoconazole (Bhaskar, 2008).

1. Pharmacokinetics

Absorption

Ketoconazole is more rapidly absorbed and produces higher concentrations in plasma when administered to infants and children as a suspension than as a poweder mixed with applesauce (Ginsburg *et al.*, 1983). Oral absorption of ketoconazole varies among individuals and bioavailability of tablet is 75% (Koch, 1983; Graybill and Drutz, 1980; Chambers, 2001). Since an acid environment is required for the dissolution of ketoconazole, bioavailability is markedly depressed in patients taking H_2- histamine receptor blocking agents such as antacid, cimetidine or proton pump inhibitors, thus should take their drugs at least 2 hours before ketoconazole (Chambers, 2001; Van der Meer *et al.*, 1980). Ingestion of food has no significant effect on the maximal concentration of the drug achieved in plasma (Daneshmend *et al.*, 1984). After oral dose of 200, 400, and 800 mg, peak plasma concentrations of ketoconazole are approximately 4, 8 and 20 µg/ml, respectively. Ketoconazole is a lipophilic with poor water solubility except at low pH (pH < 3) (Van der Meer *et al.*, 1980). Peak serum concentrations of ketoconazole occur within 1 to 4 hours (Prod Info Nizoral® tablets, 1996).

Distribution

The drug is rapidly and widely distributed throughout the body in animal and human. However, the volume of distribution was only 0.36 l/kg (Van, 1984). Ketoconazole is extensively bound in human whole blood (99%), with 84% to plasma proteins, largely albumin and 15% to erythrocytes; 1% is free (Heel *et al.*, 1982; Chambers, 2001). Ketoconazole is hightly distributed into saliva and detectable. It penetrates poorly into CSF of patients with fungal meningitis is less than 1% of the total drug concentration in plasma. In staudy, plasma protein binding of ketoconazole was altered in patients with chronic renal disease and hepatic cirrchosis, with the percentage of free ketoconazole markedly increased compared to controls (Martinez-Jorda *et al.*, 1990). Mean peak plasma levels of approximately 3.5 µg/ml are reached within 1 to 2 hours, following oral administration of a single 200 mg dose taken with a meal. Subsequent plasma elimination is biphasic with a half-life of 2 hours during the first 10 hours and 8 hours thereafter. Following absorption from the gastrointestinal tract, ketoconazole is converted into several inactive metabolites.

Elimination

Ketoconazole is extensively metabolized by hydroxylation and by oxidative *N*-dealkyllation itsleft appears to be oxidized by CYP3A. It dose not induce its own metabolism, as clotrimazole dose. The major route of elimination being as metabolites in bile (Prod Info Nizoral® tablets, 2000; Graybill and Drutz, 1980) and excreted as unchanged drug is 2% to 4% (Prod Info Nizoral® tablets, 2000). In three human

volunteers given H-keyoconazole 2.5 mg/kg about 70% of the administered dose was excreted within 4 days (57% in faece and 13% in urine). Of the faece radioactivity 20-65% was due to unchanged drug and 2-4% of urinary radioactivity (Gascoigne, *et al.*, 1981). There may be enterohepatic circulation because the double peaks plasma concentrations were seen at higher doses of ketoconazole (Brass *et al.*, 1982). Renal insufficientcy does not affect the plasma concentrations or half life, but the half-life is prolonged in patients with hepatic insufficiency.The elimination half-life appeared to be dose-dependent, increasing with increasing dose and after repeated dosing (Daneshmend *et al.*, 1983). With an oral dose of 200 mg, the range of mean ketoconazole half-life is 1.51 to 4 h. At higher dose (400 and 800 mg) the mean half-life was 3.7 h (range from 1.3 to 11.6 h) (Maksymink *et al.*, 1982).

2. Mode of Action

In vitro studies suggest that ketoconazole impairs the synthesis of ergosterol, which is a vital component of fungal cell membranes. Ketoconazole tablets are active against clinical infections with *Blastomyces dermatitidis, Candida spp., Coccidioides immitis, Histoplasma capsulatum, Paracoccidioides brasiliensis,* and *Phialophora spp.* Ketoconazole tablets are also active against *Trichophyton spp., Epidermophyton spp.* and *Microsporum spp.* Ketoconazole is also active *in vitro* against a variety of fungi and yeast. In animal models, activity has been demonstrated against *Candida spp., Blastomyces dermatitidis, Histoplasma capsulatum, Malassezia furfur, Coccidioides immitis,* and *Cryptococcus neoformans.*

3. Therapeutic use

3.1 Acne

Ketoconazole 300 mg given twice daily was effective in 3 females with acne and hirsutism. Singnificant improvement in acne occurred after 2 months of therapy. Due to the potential of hepatotoxicity with ketoconazole, topical formulation of ketoconazole should be evaluated (Ghetti *et al.*, 1986).

3.2 Arthritis

Ketoconazole has been used to treat Candida arthritis in doses of 400 to 800 mg/day; however, the efficacy of ketoconazole for this indication has been fully establishing (Silveira *et al.*, 1993).

3.3 Athlete androgen administration test

The suppressive effects of ketoconazole on testicular and androgen production demonstrated an effective test to distinguish testosterone and other androgen administration in the study involving testosterone pretreated male subjects and untreated healthy male subjects. Study participants received ketoconazole 400 mg at 0 and 2 h after sampling and were enrolled in five separate study groups. The testosterone treated subjects included the subjects receiving ketoconazole on day 3 (n

= 9), subjects receiving ketoconazole 400 mg on day 10 (n = 9), and subjects with mild primary hypogonadism (n = 5) on stable testosterone medication receiving ketoconazole on day 8. The two untreated groups administered ketoconazole on day 3, the controls (n = 9) and athletes that had been previously tested three time with a naturally high testosterone to epitestosterone ratio (T/EpiT) (n = 5). Serum testosterone and urinary T/EpiT (n = 5) ratio were evaluated for two hours for an 8 hour-time-period. A significant difference was evident between the testosterone treated groups and untreated groups (P < 0.0001). The serum testosterone concentration remained uncharged and the T/EpiT ratio increased in the testosterone treated study groups after administration of ketoconazole, where the serum testosterone concentration and T/EpiT ratio decreased by 90% and 60%, respectively, after ketoconazole administration in untreated groups. The suppressive effects of ketoconazole on endogenous androgen production support a useful and effective test for verifying testosterone and other androgen administration by athletes. In addiction, the ketoconazole suppression test may provide an opportunity to distinguish between those athletes with naturally high T/EpiT ratio and those administering testosterone (Oftebro et al., 1994).

3.4 Blastomycosis

Infections with *Blmatitastomycoses dermatitidis* are common in the Midwest and Southeastern areas in the U.S. The most common organs involved are the skin and the lung (Meyer et al., 1993). For the treatment of non-life threatening blastomycosis in immunocompetent patients, the current drug of choice is ketoconazole (Anon, 1992); however, one reference disagrees and the authors feel that itraconazole should be the drug of choice because it is more effective and better tolerated than ketoconazole (Como and Dismukes, 1994). For the treatment of more serious infections or those involving the central nervous systems, amphotericin B is considered the drug of choice (Anon, 1992; Mandell et al., 1990). For patients who are immunocompetented such as transplanted patient or patients with AIDS, treatment with amphotericin B followed by long-term treatment with ketoconazole in doses of 400 to 800 mg daily has been used (Serody et al., 1993).

3.5 Candidiasis-cutaneous

Topical 2% ketoconazole cream has been shown to produce greater drug concentrations in stratum cornium and greater bioactivity in bioassay against *C. albicans* than topical 2 % miconazole cream (Pershing et al., 1994).

3.6 Candidiasis-disseminated

For the treatment of disseminated candidiasis, amphotericin B is the drug of choice (Anon, 1992). When the used of amphotericin B is not possible, agents such as fluconazole and ketoconazole have been used successfully.

3.7 Candidiasis-esophageal

For the treatment of esophageal candidiasis, amphotericin, systemic antifungal therapy is necessary. Either fluconazole or ketoconazole are considered the drugs of choice. Dose of ketoconazole are usually 200 to 400 mg daily for 2 to 3 weeks (Anon, 1992). In patients with AIDS, maintenance therapy may be necessary to recurrence (Fauci *et al.*, 1984).

3.8 Candidiasis-oral

Ketoconazole has been effective for the treatment of oral and esophageal candidiasis. Ketoconazole 200 to 800 mg/day orally has been useful for treating severe oral or esophageal candidiasis (unresponsive to nystatin) in patients with the acquired immunodeficiency syndrome (AIDS). Since oral candidiasis in AIDS tends to recur once treatment is stopped, patients should be permanently maintained on oral nystatin or ketoconazole therapy (Fauci *et al.*, 1984)

3.9 Candidiasis-urinary

Ketoconazole was used in 11 patients to treat 13 episodes of fungal urinary infection. Eight of them were caused by Candidiasis species, 1 by mixed *C. tropicalis* and *T. glabrata*, and 3 by *T. glabrata*. Ketoconazole was administered orally at dose of 200 to 800 mg/day for courses ranging from 5 days to more than 2 years. Five episodes of the candida infections and 1 of the *T. glabrata* infections resolved in association with ketoconazole therapy (Graybill *et al.*, 1983).

3.10 Candidiasis- vaginal

Ketoconazole is not currently FDA approved for the treatment of vulvovaginal candidiasis. The CDC recommends the use of topical antifungal therapy (CDC, 1993). However, in several trials oral ketoconazole has been effective for the treatment of vaginal candidiasis (Talbot and Spencer, 1993; Kovacs *et al.*, 1990; Balbi *et al.*, 1986; Sobel, 1986).

4. Warning

When used orally, ketoconazole has been associated with hepatic toxicity, including some fatalities. Patients receiving this drug should be informed by the physician of the risk and should be closely monitored.

Coadministration of terfenadine with ketoconazole tablets is contraindicated. Rare cases of serious cardiovascular adverse events, including death, ventricular tachycardia and tordades de pointes have been observed in patients taking

ketoconazole tablets concomitantly with terfenadine, due to increased terfenadine concentrations induced by ketoconazole tablets. Pharmacokinetic data indicate that oral ketoconazole inhibits the metabolism of astemizole, resulting in elevated plasma levels of astemizole and its active metabolite desmethylastemizole which may prolong QT intervals. Coadministration of astemizole with ketoconazole tablets is therefore contraindicated.

Coadministration of cisapride with ketoconazole is contraindicated. Serious cardiovascular adverse events including ventricular tachycardia, ventricular fibrillation and torsades de pointes have occurred in patients taking ketoconazole concomitantly with cisapride.

Hepatotoxicity, primarily of the hepatocellular type, has been associated with the use of ketoconazole tablets, including rare fatalities. The reported incidence of hepatotoxicity has been about 1:10,000 exposed patients, but this probably represents some degree of under-reporting, as is the case for most reported adverse reactions to drugs. The median duration of ketoconazole tablets therapy in patients who developed symptomatic hepatotoxicity was about 28 days, although the range extended to as low as 3 days. The hepatic injury has usually, but not always, been reversible upon discontinuation of ketoconazole tablets treatment. Several cases of hepatitis have been reported in children.

Prompt recognition of liver injury is essential. Liver function tests (such as SGGT, alkaline phosphatase, SGPT, SGOT and bilirubin) should be measured before starting treatment and at frequent intervals during treatment. Patients receiving ketoconazole tablets concurrently with other potentially hepatotoxic drugs should be carefully monitored, particularly those patients requiring prolonged therapy or those who have had a history of liver disease.

Most of the reported cases of hepatic toxicity have to date been in patients treated for onychomycosis. Of 180 patients worldwide developing idiosyncratic liver dysfunction during ketoconazole tablets therapy, 61.3% had onychomycosis and 16.8% had chronic recalcitrant dermatophytoses.

Transient minor elevations in liver enzymes have occurred during treatment with ketoconazole tablets. The drug should be discontinued if these persist, if the abnormalities worsen, or if the abnormalities become accompanied by symptoms of possible liver injury.

In rare cases anaphylaxis has been reported after the first dose. Several cases of hypersensitivity reactions including urticaria have also been reported.

Coadministration of ketoconazole tablets and terfenadine has led to elevated plasma concentrations of terfenadine which may prolong QT intervals, sometimes resulting in life-threatening cardiac dysrhythmias. Cases of torsades de pointes and other serious ventricular dysrhythmias, in rare cases leading to fatality, have been

reported among patients taking terfenadine concurrently with ketoconazole tablets. Coadministration of ketoconazole tablets and terfenadine is contraindicated.

Concomitant administration of ketoconazole tablets with cisapride is contraindicated because it has resulted in markedly elevated cisapride plasma concentrations and prolonged QT interval, and has rarely been associated with ventricular arrhythmias and torsades de pointes. In European clinical trials involving 350 patients with metastatic prostatic cancer, eleven deaths were reported within two weeks of starting treatment with high doses of ketoconazole tablets (1200 mg/day). It is not possible to ascertain from the information available whether death was related to ketoconazole therapy in these patients with serious underlying disease. However, high doses of ketoconazole tablets are known to suppress adrenal corticosteroid secretion.

In female rats treated three to six months with ketoconazole at dose levels of 80 mg/kg and higher, increased fragility of long bones, in some cases leading to fracture, was seen. The maximum "no-effect" dose level in these studies was 20 mg/kg (2.5 times the maximum recommended human dose). The mechanism responsible for this phenomenon is obscure. Limited studies in dogs failed to demonstrate such an effect on the metacarpals and ribs.

5. Drugs Interactions

5.1 Oral Anticoagulants

A patient had been treated with warfarin for three years for apulmonary Embolism, and later recived ketoconazole 200 mg twice daily for chronic vaginal thrush infection. After three weeks of treatments with ketoconazole she complained of subcutaneous bruising and reported to the clinic, whereas platelet count and liver function tests gave normal results. Treatment of ketoconazole was stoped, warfarin dosage reduced. Over the next three weeks her warfarin control was re-established at previous level (Smith, 1984). Brass *et al.*, (1982) found no hypoprothrombinemic interaction in two volunteers receiving 200 mg ketoconazole plus 7.5 mg to 15 mg warfarin for three weeks.

5.2 Benzodiazepines

Chlordiazepoxide is extensively oxidized in the liver with little urinary excretion of the parent drug. Ketoconazole impair of distributioned chlordiazepoxide clearance from plasma. After a single dose of ketoconazole there was a 20% decrease in clearance and 26% decrease in volume without evidence of inhibition of drug metabolism. These changes apparently were not related to ketoconazole dose. After repetitive dosing with ketoconazole, chlordiazepoxide clearance decreased by 38% and was associated with reduced concentrations of its first oxidative metabolite, N-

desmethy chlordiazepoxide. It was concluded that ketoconazole inhibits at least one subset of the hepatic mixed-function oxidase system, but not generally (Brown *et al.*, 1985)

Concomitant use of ketoconazole and alprazolam may results in increased serum concentrations of alprazolam toxicity (excessive sedation, fatigue, ataxia, slurred, slowed reactions and other psychomotor impairment). *In vitro* studies have shown ketoconazole to be a potent inhibitor of cytochrome P450 3A (CYP3A) enzymes, an enzyme subfamily thought to be important in alprazolam metabolism (von Moltke *et al.*, 1994 ; Greenblatt *et al.*, 1993; Greenblatt *et al.*, 1998). Because the initial step in alprazolam metabolism is hydroxylation catalyzed by CYP3A, ketoconazole may have a profound effect on the clearance of alprazolam. Concomitant administration of these two agents is contraindicated (Fenneteau *et al.*, 2009).

Triazolam is a short-acting hypnotic having an average $t_{1/2}$ of 2 to 4 hours. After oral administration, triazolam is metabolized during its absorption (first-pass) and elimination phase by CYP3A4. Nine healthy volunteers received 400 mg ketoconazole, 200 mg itraconazole, or matched placebo orally once a day for 4 days. On day 4, each ingested a single 0.25 mg dose of triazolam. Ketoconazole and itrazonazole increase AUC of triazolam by 22-fold and 27-fold, C_{max}, by 3-fold, and $t_{1/2}$ by 6-fold and 7-fold, respectively. All pharmacodynamic effects revealed a significant difference between the antimycotics and placebo phases. Ketoconazole and itraconazole seriously affects the pharmacokinetic of triazolam and increase the intensity and duration of its effects by inhibition of CYP3A4 during the absorption and elimination phases of triazolam (Varhe *et al.*, 1994).

The role of P-glycoprotien (P-gp) on the distribution of the benzodiazepine, triazolam, and the azole antifungal agent ketoconazole, and on the triazolam-ketoconazole infection, was studies using mdr la (-) or mdr la/b (-/-) mice (P-gp-deficient mice) and matched controls. Compared with animals receiving triazolam alone, co-administration of triazolam with ketoconazole caused an elevation of serum, brain, and liver concentrations of triazolam. This was true in both FVB controls and mdr la (-) mice. Likewise, liver/serum ratios were not significantly changed by co-treatment of ketoconazole (von Moltke *et al.*, 2004).

Substantial increases in oral midazolam peak plasma concentration (310%), AUC (1490%) and half–life (210%) have been demonstrated to occur with concurrent oral ketoconazole compared to placebo in healthy volunteers (Olkkola *et al.*, 1994). Psychomotor tests and subjective reporting of drowsiness with the combination indicated significant increases in sedative. Oral midazolam is not recommended for patients receiving ketoconazole. Ketoconazole is known inhibitor of the cytochrome P450 3A4 (CYP3A4) enzyme system, and midazolam metabolism is mediated through CYP3A4. Co-administration of these two agents may result in prolonged sedation due to reduced midazolam plasma clearance (Prod Info Versed®, 1997).

Inhibited CYP3A4 activity caused by ketoconazole appears to be greater in the intestine than in the liver (Tsunoda *et al.*, 1999).

5.3 Calcium channel blocking agents

Ketoconazole inhibits hepatic cytochrome isoenzyme CYP3A4 (Pro Info Nizoral®, 1998), an enzyme involved in the metabolism of some dihydropyridine calcium channel antagonists including nifedipine, nicardipine, amiodipine, isradipine and felodipine (Guengerich *et al.*, 1991; Josefsson *et al.*, 1996). Pre-treatment with and concomitant administration of ketoconazole resulted in a 24-fold, and 11 fold increase in mean AUC and C_{max} of nisoldipine, respectively, compared with treatment with nisoldipine 5 mg alone (Heinig *et al.*, 1999). Peripheral edema and elevated calcium antagonist serum concentrations during concurrent use of itraconazole and felodipine, isadipine, or nifedipine (Neuvonen and Suhonen, 1995; Tailor *et al.*, 1996). Since the other triazole and imidazole antifungals antifungals also inhibit CYP3A4, this interaction would be expected to occur with other combinations.

5.4 Amphotericin

Animal studies and *in vitro* investigations have found antagonism between amphotericin B and azole antifungal derivatives.The mechanism of action of azoles is inhibit ergosterol synthesis in fungal cell membranes. Amphotericin B acts by binding to sterols in the cell membrane and changing membrane permeability. Clinical effects of this antagonism are to date unknown (Prod Info Abelcet®, 1999).

5.5 Trilazad

Trilazad mesylate (U-89678) is a membrane lipid peroxidation inhibitor that shows efficacy in reducing the damaging effects of lipid peroxidation on the cell membrane triggered by brief preriod of ischemia. Trilazad is highly metabolized after intravenous administration in healthy volunteers. It was postulated that the limited bioavailability was due to extensive first-pass metabolism in the liver. The major pathways of tirilazad metabolism in man are mediated by the CYP3A. Pretreatment with ketoconazole for 7 days results in increased mean U-89678 AUC by 67% and 309% for intravenous and oral administration, respectively. Mean AUC for U-89678 were increase 472% and 720% by ketoconazole administration with i.v. and oral tirilazad, respectively, whereas increases of more than 10-fold in mean U-87999 (another active metabolites) AUC. Ketoconazole increased the bioavailability 20.9% by decreasing the first-pass liver and gut wall metabolism of trilazad mesylate in similar degrees. These results indicate that ketoconazole inhibits the metabolism of three compounds (Tirilazad, U-89678 and U-87999), which suggests that all of the compounds are substrates for CYP3A (Fleishaker *et al.*, 1996).

5.6 Quinine

Mirghani *et al.* (1999) showed the effect of ketoconazole on quinine pharmacokinetics, it (which inhibit CYP3A4) significantly deceased the mean apparent oral clearance of quinine by 31%, whereas co-administration with fluvoxamine (which inhibits CYP1A2 and to some extent CYP2C19) had no significants effect on the mean apparent oral clearance of quinine. Co-administration with fluvoxamine increase 3-hydroxyquinine *in vivo*. On the other hand, CYP1A2 had no significant effect on this metabolic pathway.

5.7 Quinidine

Ketoconazole is well known to inhibit potently CYP3A4 activity. However, ketoconazole inhibits competitively the metabolic activity of CYP3A12, a major isoenzyme of CYP3A subfamily in canine hepatic microsomes (Kuroha *et al.*, 2004). Quinidine (QN) was used as a model drug of CYP3A12 substrate. The result shown that higher concentration of QN was observed after than before ketoconazole treatment. The pharmacokinetic parameters of QN estimated by two-compartmental method, when co-administration of ketoconazole, statistically significant was observed in an exponential at distribution phase (α), at elimination phase (β), $t_{1/2\,\beta}$, V_1, and k_a of QN phase. The pharmacokinetic parameters of QN estimated by non-compartmental method, when co-administration of ketoconazole, Cl_{tot}, Cl_{oral}, C_{max}, $MRT_{i.v.}$, $MRT_{p.o.}$, MATwere statistically significant of QNphase (Kuroha *et al.*, 2004).

5.8 Reboxetine

Reboxetine is a specific norepinephrine reuptake inhibitor that is licensed in several European countries for treatment of depression. It is metabolized by CYP3A4. Eleven healthy volunteers received 4 mg reboxetine orally on the 2nd day of a 5 days regimen of 200 mg ketoconazole once daily in a crossover design. Ketoconazole increased R, R (-)- reboxetine (more active reboxetine enantiomers) mean AUC by 58% and 43%, respectively ($P<0.02$). Oral clearance of both enantiomers was consequently decreased 34% and 24%, respectively by ketoconazole ($P<0.05$). Mean terminal half-life after administration of ketoconazole (21.5 and 18.9 h) was significantly longer than after reboxetine alone (14.8 and 14.4 h; P, 0.005).The AUC ratio for R, R (-)-reboxetine was reduced by ketoconazole administration (12.76 after ketoconazole versus 2.39; $p<0.003$).

Ketoconazole decreased clearance of both reboxetine enantiomers. Although the adverse effect profile for reboxetine was not altered by ketoconazole, the results of this study suggest that caution should be taken and that a reduction in reboxetine dose should be considered when the two drugs are co-administered (Herman *et al.*, 1999).

5.9 Amprenavir

Twelve individuals received single dose of amprenavir 1200 mg and ketoconazole 400 mg. Maximum concentration (C_{max} of amprenavir was decreased an average of 16%, but the area under the concentration-time curve (AUC) increased 31%. Amprenavir increased the ketoconazole C_{max} and AUC by 19% and 44%, respectively. The significant of this interaction is unknown, but unlikely to be clinically important (Polk *et al.*, 1999).

5.10 Tolbutamide

A study was carried out to find out the therapeutic implication of the tolbutamide-ketoconazole interaction in diabetic rabbits. Ketoconazole treatment (20 mg/kg, oral once daily for one week) significantly increased and prolonged the antidiabetic effect of tolbutamide (40 mg/kg, oral) in diabetic rabbits. The AUC and the terminal half-life of tolbutamide were increased significantly by ketoconazole indicating the decrease in its hepatic metabolism (Krishnaiah, *et al.*, 1993).

5.11 Antihistamine

Enzyme inhibiting drug such as ketoconazole may lead to high level of astemizole if used concurrently. Astemizole overdoses have led to prolonged QT interval and severe ventricular arrhythmias (Hoppu., *et al.*, 1991; Snook., *et al.* 1988). Due to the potential for an interaction that could lead to increased astemizole use with ketoconazole is contraindicated (Keogh and Kunta, 2006).

5.12 Antacids

Ketoconazole can be administered with some antacids that could modify its dissolution rate and reduce its absorption leading to therapeutic failures. Concurrent administration of ketoconazole with antacids, cimetidine or sodium bicarbonate has resulted in both a decrease in plasma peak concentration and the AUC of ketoconazole (Brass *et al.*, 1982; Carlson *et al.*, 1983).

5.13 Ritonavir and Saquinavir

Ketoconazole significantly increased area under the plasma concentration time curve, plasma concentration at 12 hours after the dose, and half-life of ritonavir by 29%, 13%, 62% and 31%, respectively. Similar increases of 37%, 94% and 38%, respectively, were observed for these parameters for saquinavir. Ketoconazole significantly elevated ritonavir CSF concentration by 178%, from 2.4 to 6.6 ng/ml, with no change in paired unbound plasma level (26 ng/ml) ; this led to a commensurate 181% increase in CSF/plasma unbound ratio, but not significant for saquinavir. The disproportionate increase in CSF compared with plasma concentrations of ritonavir is consistent with ketoconazole inhibiting both drug efflux from CSF and systemic clearance (Khaliq *et al.*, 2000).

5.14 Oestrogen

Oestrogen undergoes extensive oxidative metabolism by cytochrome P450 enzymes. A major route is hydroxylation.The hydroxylation of oestrone is reported to be catalyzed primarily by CYP3A4. Annas *et al.* (2003) showed the effect of ketoconazole on oestrogen metabolism in postmenopausal women; it significantly increased the mean AUC of oestrogen (+4029 $nmoll^{-1}h$) and its C_{max} (+306 $nmol \, l^{-1}$).

5.15 Cocaine and opioid

In studies with rodents ketoconazole decreased both the rate of acquisition of cocaine self-administration and the percentage of meeting the acquisition criterion but only under food-restricted condition. In contrast, studies in primates and humans have produced conflicting results using cortisal synthesis inhibitors for attenuating cocaine-related behaviors and subjective effects. To expolore the treatment implications of these finding, ketoconazole's (600-900 mg daily) ability to reduce heroin and cocaine use was compared with placebo in 39 methadone maintained patients with a history of cocaine abuse or dependence during a 12-week double blind trail. Contrary to the predicted effects, both heroin and cocaine use increased after patients were stabilized on methadone and ketoconazole. Depressive and withdrawal symptoms improved no more with ketoconazole than with placebo treatment, and side effects were greater on ketoconazole than placebo. As reported before with methadone treatment, morning cortisol levels were significantly lower than normal values throughout the clinical trail, but were not lower with ketoconazole than placebo treatment. Thus, in agreement with the negative results from acute dosing studies in primates and humans, chronic ketoconazole treatment does not appear to reduce cocaine or opioid use in humans maintained on methadone (Kosten *et al* ., 2002).

The co-administration with ketoconazole could increases plasma concentration of Levo-acetyl-α-methadol (LAAM), a synthetic opioid, use in replacement maintenance therapy for opioid dependence. The long duration of action of LAAM has been attributed primarily to its sequential *N*-demethylation to two primary metabolites, norLAAM and dinor LAAM, which have more potent α-agonist activity. The *N*-demethylation of both LAAM and norLAAM is performed primary by cytochrome P450 (CYP) 3A4, with contributions from CYP2B6, CYP3A5, and members of the CYP2C family (8, 9, 18, and all posses some activity). CYP3A4 substrates are offen subject to in vivo drug interactions. The most dramatic effects of ketoconazole were the increase in the AUC (5.29-fold) and C_{max} (3.22-fold) of LAAM, along with the extension of the t_{max} of norLAAM (2.43-fold) and dinorLAAM (11.6-fold). The increase in LAAM concentration occurred without any significant effect on its t_{max} or $t_{1/2}$.For norLAAM and dinorLAAM, the extension of the t_{max} was accompanied by decreased C_{max}, increase AUC, and slightly increased $t_{1/2}$. The urinary excretion of LAAM, norLAAM, and dinorLAAM paralleled the plasma concentration when LAAM was given after placebo or ketoconazole (Moody *et al.*, 2004).

5.16 Cyclosporine

Five male renal allograft patients of mean age 22 ± 2.3 years displayed a mean AUC of cyclosporine before strting ketoconazoloe to be 1581 ± 484 ng.ml/h. Following addition of 50 mg of ketoconazole, the mean AUC of cyclosporine increased to 4946 ± 1006 ng.ml/h ($P<0.01$) (Abraham *et al.*, 2003).

5.17 Antiarrhythmic drug

Azimilide dihydrochloride is aclass III antiarrhythmic drug. It was metabolized by CYP 3A4/5 and CYP 1A1. Pretreatment with ketoconazole decreased azimilide oral clearance (14%), and increases azimilide C_{max} (120%), AUC (16%), and $t_{1/2}$ (13%). No change in t_{max}, V_Z/F or plasma protein binding was observed (Mouelhi *et al.*, 2004).

5.18 Immunosuppressant

Everolimus is a macrolide immunosuppressant indicated for prevent of acute rejection episodes after kidney and heart transplantation. It is extensively metabolized by CYP3A to form inactive metabolites that are eliminated in the bile. It is also a substrate of the drug efflux transporter P-glycoprotein. Co-administration of ketoconazole with everolimus can increase everolimus C_{max}, AUC, and $t_{1/2}$ to 3.9, 15 and 1.9 folds (Kovarik *et al.*, 2005).

5.19 Retinoic acid

The CYP inhibitors (diethyl dithiocarbamate DEDTC, ketoconazole (KC) and grapefruit juice (GJ) could change the pharmacokinetics of All-trans-retinoic acid (ATRA). DEDTC was a powerful inhibitor of ATRA metabolism, with an apparent IC_{50} value of 0.12 μM, whereas the apparent TC50 value for ketoconazole (KC), a well-established CYP3A4 inhibitor, was 13.5 μM. On the other hand, grapefruit juice (GJ) has been reported to increase the bioavailability of certain drugs by inhibiting the intestinal CYP enzyme. For infusion of ATRA, the co-administration of i.v. or oral DEDTC did not change the profile of the plasma AUC of ATRA. However, co-administration of oral KC yielded higher plasma ATRA levels than those obtained in the control group and, consequently, higher C_{max} and AUC $_{0-600}$ values were obtained for the rats in this group. For oral administration of ATRA, after the oral administration of 6.4 and 32 mg/kg of DEDTC, no changes in ATRA pharmacokinetics were observed since the plasma level profile and parameters values ATRA were similar to those obtained in the control group. However, after the oral administration of 320 mg/kg of DEATC, a pronounced decrease in plasma ATRA was recorded, and the mean C_{max} and $AUC_{0-\infty}$ values were approximately 20% and 24% of the corresponding mean values obtained in the control group. The oral administration of KC gave rise to an increase in the plasma levels, $AUC_{0-\infty}$ and $t_{1/2}$ values of ATRA,

whereas the oral administration of GJ did not affect the pharmacokinetics of orally administered ATRA (Saadeddin *et al.*, 2004).

5.20 Antidiabetic drug

Ketoconazole increased the plasma concentrations of rosiglitazone. After administration of ketoconazole, the mean $AUC_{0-\infty}$ of rosiglitazone was increased by 47%. The elimination half-life of rosiglitazone was lengthened from 3.55 h to 5.50 h and C_{max} was also increased by 17%. The Cl/F decreased about 30% after ketoconazole treatment compared with placebo treatment, Ketoconazole is well known to inhibit potently CYP3A4 activity but rosiglitazone is metabolized through *N*-demethylation and *p*-hydroxylation, mainly by CYP2C8 and to a lesser extent CYP2C9.Therefore, this study revealed that ketoconazole affected the disposition of rosiglitazone in humans, probably by the inhibition of CYP2C8 and CYP2C9 (Park *et al.*, 2004).

6. DOSAGE AND ADMINISTRATION

The recommended starting dose of ketoconazole tablets is a single daily administration of 200 mg (one tablet). In very serious infections or if clinical responsiveness is insufficient within the expected time, the dose of ketoconazole tablets may be increased to 400 mg (two tablets) once daily.

In small numbers of children over 2 years of age, a single daily dose of 3.3 to 6.6 mg/kg has been used. Ketoconazole tablets have not been studied in children under 2 years of age.

There should be laboratory as well as clinical documentation of infection prior to starting ketoconazole therapy. Treatment should be continued until tests indicate that active fungal infection has subsided. Inadequate periods of treatment may yield poor response and lead to early recurrence of clinical symptoms. Minimum treatment for candidiasis is one or two weeks. Patients with chronic mucocutaneous candidiasis usually require maintenance therapy. Minimum treatment for the other indicated systemic mycoses is six months.

Minimum treatment for recalcitrant dermatophyte infections is four weeks in cases involving glabrous skin. Palmar and plantar infections may respond more slowly. Apparent cures may subsequently recur after discontinuation of therapy in some cases.

7. Factors Affecting the Pharmacokinetics of Ketoconazole

Influence of Food Intake

Food has not been reported to reduce ketoconazole absorption or significantly alter peak levels. However, there is a food-related delay in achieving peak concentrations (Daneshmend *et al.*, 1984).

Renal Insufficiency

Dose reductions are not required in patients with renal failure, since very little active drug is excreted via the kidneys (Graybill and Drutz, 1980; Heel *et al*., 1982).

Hepatic Insufficiency

Ketoconazole is extensively metabolized in the liver. However, specific dosing adjustments have not been described (Graybill and Drutz, 1980). Dose reduction should be considered in patients with severe liver disease.

8. Adverse reactions

In rare cases, anaphylaxis has been reported after the first dose. Several cases of hypersensitivity reactions including urticaria have also been reported. However, the most frequent adverse reactions were nausea and/or vomiting in approximately 3%, abdominal pain in 1.2%, pruritus in 1.5%, and the following in less than 1% of the patients: headache, dizziness, somnolence, fever and chills, photophobia, diarrhea, gynecomastia, impotence, thrombocytopenia, leukopenia, hemolytic anemia, and bulging fontanelles. Oligospermia has been reported in investigational studies with the drug at dosages above those currently approved. Oligospermia has not been reported at dosages up to 400 mg daily, however sperm counts have been obtained infrequently in patients treated with these dosages. Most of these reactions were mild and transient and rarely required discontinuation of ketoconazole tablets. In contrast, the rare occurrences of hepatic dysfunction require special attention.

In worldwide postmarketing experience with ketoconazole tablets there have been rare reports of alopecia, paresthesia, and signs of increased intracranial pressure including bulging fontanelles and papilledema. Hypertriglyceridemia has also been reported but a causal association with ketoconazole tablets is uncertain.

Neuropsychiatric disturbances, including suicidal tendencies and severe depression, have occurred rarely in patients using ketoconazole tablets

Ventricular dysrhythmias (prolonged QT intervals) have occurred with the concomitant use of terfenadine with ketoconazole tablets. Data suggest that

coadministration of ketoconazole tablets and cisapride can result in prolongation of the QT interval and has rarely been associated with ventricular arrhythmias.

9. Overdosage

In the event of accidental overdosage, supportive measures, including gastric lavage with sodium bicarbonate, should be employed.

III. Drug metabolism

Drugs are eliminated either unchanged by the process of excretion or via biotransformation to metabolites. Most drugs are lipid-soluble, which promotes their passage through biological membranes and enables access to their site of action. Most lipophilic compounds are, however, eliminated poorly unless they are metabolized to more polar compounds (Meyer, 1996). The metabolites are usually inactive or less active than the parent drug. However, some metabolites may have enhanced activity (prodrugs) or toxic effects.

Drug biotransformation reactions can be classified into phase I functionalisation reactions or phase II conjugation reactions. Phase I reactions introduce a functional group on the parent compound by oxidation, reduction or hydrolysis reactions, many of which are catalysed by the CYP system and require NADPH as a cofactor. Phase II reactions lead to the formation of a covalent linkage between a functional group of the parent drug or phase I metabolite and an endogenous compound and include glucuronidation, sulfation, acetylation and methylation reactions. The enzyme systems involved in phase I reactions are located mainly in the endoplasmic reticulum, while phase II conjugating enzymes are located in both the cytoplasm and the endoplasmic reticulum (Krishna and Klotz, 1994).

The liver is the principal organ of drug metabolism, although other organs, such as the gastrointestinal tract, kidneys, lung and skin, can have significant metabolic capacity (Krishna and Klotz., 1994). A notable portion of a drug may be metabolised in the intestine or liver before entering the systemic circulation, which can significantly limit the oral bioavailability of a drug (first-pass metabolism).

Metabolic capacity can vary markedly between individuals, leading to differences in drug response and adverse effects among patients (Wilkinson, 2005). The variability in metabolic capacity is multifactorial; gender, polymorphism of drug-metabolising enzymes, smoking, dietary factors and other drugs can all affect drug metabolism.

1. CYP enzyme system
Overview

The cytochromes P450, CYP enzymes, are a superfamily of heme-containing enzymes, of which over 2700 individual members are currently known to exist in nature. (Lewis, 2004). They have been named after their characteristic absorption wavelength maximum (450 nm), seen when the reduced form of the enzyme is bound to carbon monoxide (Omura et al., 1962). The CYPs are able to metabolise a diverse group of substrates, both and xenobiotic and endogenous such as arachidonic acid and

eicosanoids, steroid hormones, cholesterol and bile acid, vitamin D and retinoic acid (Nebert and Russell, 2002) that most often by catalysing oxidative reactions that play a major role in phase I metabolism in humans (Wrighton and Stevens, 1992).

Structure and tissue distribution

The function of CYP enzymes is based on the catalytically active center of the protein, formed around a heme.(Fontana et al., 2005) The overall sequence variability among CYP proteins is great, but they have a high conservation of their central structure and general topography.(Peterson et al., 1998) The most variable regions are associated with anchoring to membrane, and substrate binding and recognition. The substrate-recognition sites are flexible and move upon substrate binding to facilitate the catalytic process.(Gotoh, 1992) CYPs are anchored on the outer face of the endoplasmic reticulum (ER) by hydrophobic anchors, with the active site exposed at the cytosolic face of the membrane.(Cribb et al., 2005).
The CYP system is arguably our most important xenobiotic metabolizing system, and approximately 80-90% of human drug metabolism is CYP-mediated (Rendic S, 2002; Lewis, 2000; Riley, 2001).

The functions of CYPs are extremely diverse-from biosynthesis and catabolism of signalling molecules and steroid hormones to detoxification or activation of xenobiotics and they can be found in all types of tissues, with developmentally regulated patterns of expression (Mansuy, 1998). The highest levels of CYPs are found in the liver, where they were first described (Klingenberg M, 1958). The small intestine has the second highest CYP content, (Zhang et al., 1999) and CYPs are present in lower quantities in many other tissues, such as the kidney, brain, and the respiratory tract (Ding et al., 2003).

In the liver, CYP3A4 is quantitatively the most important, with CYP2C8, CYP2C9, CYP2A6, CYP2E1 and CYP1A2 present in somewhat lower quantities; CYP2C19 and CYP2D6 are of relatively minor quantitative importance, but their clinical importance is high (Shimada et al., 1994) CYP3A4 is the major form of CYP expressed in enterocytes, and members of CYP2C subfamily are also significantly expressed (Zhang et al., 1999). Microsomal protein content decreases along the small intestine from the duodenum to ileum (Obach et al., 2001).

Catalytic mechanism

CYPs most often catalyse the insertion of one of the atoms of molecular oxygen into the substrate being biotransformed, while the second atom of oxygen is reduced to water. The most frequently catalysed reaction is hydroxylation, but the result of CYP-mediated catalysis can be e.g. a dealkylation, dehydrogenation, isomerisation or carbon-bond cleavage.(Mansuy, 1998) The variety of CYP enzyme structures and the intrinsic reactivity of all their substrates explains the diversity of reactions catalysed by CYPs (Werck-Reichhart et al., 2000). The details of the mechanism by which CYPs carry out all types of reactions are not fully understood. The best-documented aspect is the hydroxylation reaction that is common to most CYPs.

Substrate selectivity

The CYPs have broad and often overlapping substrate specificities. It is possible that two or more CYPs contribute to the metabolism of a single compound, or that a single CYP can catalyse two or more metabolic reactions for the same substrate. Although more than one CYP can catalyse the biotransformation of a drug, they may do so with markedly different affinities. The selectivity of a substrate towards a particular CYP enzyme is based on differences in the number and specie disposition of the relevant complementary structures on both substrate and enzyme molecules. The lipophilic character and molecular mass of the substrate also play a role in substrate recognition. Drug biotransformation in vivo is often determined by the CYP with the highest affinity for the drug. Thus, the rate of elimination of drugs can be largely determined by a single CYP enzyme or a combination of specific CYPs (Pelkonen *et al.*, 1998) (Lewis, 2004).

2. CYP family

In humans, 57 cytochrome P450 genes arranged in 18 families have been identified, of which only the CYP1, CYP2 and CYP3 families seem to contribute to the metabolism of drugs (Nebert and Russell, 2002). CYP families are further divided into subfamilies and specific isoenzymes. All isoenzymes in the same family have at least 40% amino acid similarity, and those in the same subfamily have at least 55% amino acid similarity. Individual CYP enzymes are designated by a family number (e.g. CYP2C8), a subfamily letter (CYP2C8) and a number for an individual enzyme within the subfamily (CYP2C8) (Nelson *et al.*, 1996).

Many *in vitro* studies of CYP-mediated drug metabolism in humans are conducted using human liver microsomes (HLM). Upon homogenisation and centrifugationof liver tissue, the endoplasmic reticulum is fragmented to microvesicles, which are referred to as microsomes. The microsomes contain several drug-metabolising enzymes, including the CYP enzymes, flaving-containing mono-oxygenases (FMO) and UDP-glucuronosyltransferases (Venkatakrishnan *et al.*, 2001)

1. CYP1A subfamily

There are two members of the CYP1A subfamily in humans: CYP1A1 and CYP1A2. CYP1A1 is found primarily in extrahepatic tissues, most notably in the lung and placenta (Miners and McKinnon, 2000). CYP1A2 is mainly a hepatic enzyme, and it accounts for about 12-18% of all CYP enzymes in the liver (Rowland-Yeo *et al.*, 2004, Klein *et al.*, 2006). CYP1A enzymes are inducible by xenobiotics, such as polycyclic aromatic hydrocarbons (PAH) found in cigarette smoke and grilled food, and the induction is mainly mediated by the aryl hydrocarbon receptor (AhR) (Nebert and Russell, 2002). Rifampicin has increased, for example, the clearance of CYP1A2 substrate mexiletine by over 60%, but compared with CYP3A4, CYP1A2 seems to be only weakly induced by rifampicin in humans (Backman *et al.*, 2006).

CYP1A2 is responsible in the metabolism of many drugs, including caffeine, clozapine, theophylline and tizanidine (Bertz and Granneman, 1997, Miners and McKinnon, 2000, Granfors *et al.*, 2004). Fluvoxamine and ciprofloxacin are strong inhibitors of CYP1A2 *in vivo* (Bertz and Granneman, 1997). Furafylline is used as a selective CYP1A2 probe inhibitor *in vitro* (Miners and McKinnon, 2000). Marked

interindividual variability has been reported in the activity of CYP1A2 in humans (Miners and McKinnon, 2000). Although the CYP1A2 gene shows structural polymorphism, its importance in explaining variability in CYP1A2 activity is unclear. (www.cypalleles.ki.se/cyp1A2.htm).

2. CYP2 subfamily

CYP2A6 is the first form of human CYP enzyme in the CYP2 family. CYP2A6 is a predominantly hepatic enzyme, and it constitutes approximately 4-8% of the total liver CYP content (Rowland-Yeo et al., 2004, Klein et al., 2006). Coumarine and nicotine are specific substrates of CYP2A6, and methoxsalen is a potent inhibitor of CYP2A6 (Pelkonen et al., 2000). CYP2A6 is highly polymorphic and its genotype has been associated with, for example, smoking habits (Malaiyandi et al., 2005). CYP2A6 may be inducible by antiepileptic drugs (Pelkonen et al., 2000).

CYP2B6

CYP2B6 has recently received more attention as a clinically important enzyme in drug metabolism (Turpeinen et al., 2006). It is highly polymorphic (Lang et al., 2001), and it may represent up to 6% of the total CYP content in the liver (Stresser and Kupfer, 1999). It is expressed at lower levels in some extrahepatic tissues (Ekins and Wrighton, 1999). Clinically used substrate drugs for CYP2B6 include bupropion, cyclophosphamide, propofol, nevirapine and efavirenz (Hesse et al., 2000, Court et al., 2001, Ward et al., 2003, Turpeinen et al., 2006). CYP2B6 is inducible by, for example, rifampicin (Loboz et al., 2006). Clopidogrel and ticlopidine are potent inhibitors of CYP2B6 (Richter et al., 2004, Turpeinen et al., 2004, Turpeinen et al., 2005).

CYP2C subfamily

The human CYP2C subfamily comprises four members: CYP2C8, CYP2C9, CYP2C18 and CYP2C19. Of these, CYP2C8, CYP2C9 and CYP2C19 are of clinical importance and are collectively responsible for the metabolism of about 20% of clinically used drugs (Ingelman-Sundberg, 2004, Totah and Rettie, 2005). CYP2C enzymes are expressed mainly in the liver, where they account for approximately 20% of the total CYP content (Shimada et al., 1994), but they are expressed at a significant level also in the small intestine (Obach et al., 2001). Each member of the CYP2C subfamily is genetically polymorphic (Daly, 2003).

The importance of CYP2C8 in drug metabolism is being increasingly recognised, and it has a major role in the metabolism of a growing number of substrates, including paclitaxel, repaglinide, rosiglitazone, pioglitazone, cerivastatin, amiodarone, amodiaquine, chloroquine and arachidonic acid (Eckland and Danhof, 2000, Totah and Rettie, 2005). Some overlapping substrate specificity appears to exist between CYP2C8 and CYP3A4 in, for instance, the metabolism of carbamazepine, cerivastatin and repaglinide (Ong et al., 2000, Totah and Rettie, 2005). CYP2C8 constitutes about 7% of total microsomal CYP content in the liver, and CYP2C8 protein has been detected in several extrahepatic tissues as well (Enayetallah et al,. 2004, Totah and Rettie, 2005). CYP2C8 is inducible by rifampicin, phenobarbital and dexamethasone in vitro (Gerbal-Chaloin et al., 2001, Madan et al., 2003). Montelukast is a very selective and potent CYP2C8 inhibitor in vitro (Walsky et al.,

2005b). Gemfi brozil (gemfi brozil glucuronide) and trimethoprim inhibit CYP2C8 both *in vitro* and *in vivo* (Backman *et al.*, 2002, Wang *et al.*, 2002, Wen *et al.*, 2002, Niemi *et al.*, 2003b, Niemi *et al.*, 2003c, Niemi *et al.*, 2004a, Ogilvie *et al.*, 2006). Although the most common variant alleles CYP2C8*2 and CYP2C8*3 show decreased enzyme activity in vitro, the clinical consequences of the polymorphism have not been fully determined (Totah and Rettie, 2005).

CYP2C9 is the predominant CYP2C enzyme in both the intestine and the liver. CYP2C9 is estimated to be responsible for the metabolism of up to 15% of all drugs that undergo phase I metabolism, and its substrates include S-warfarin, phenytoin, losartan, fluvastatin, sulphonylurea antidiabetic drugs and several NSAIDs (Rettie and Jones, 2005). Sulfaphenazole is a selective and potent CYP2C9 inhibitor (Miners and Birkett, 1998). Clinically significant inhibition may occur also with coadministration of amiodarone, fluconazole, miconazole, voriconazole and certain other sulphonamides (Miners and Birkett, 1998, Th euretzbacher *et al.*, 2006). CYP2C9 activity in vivo is inducible by rifampicin (Miners and Birkett, 1998). Genetic polymorphism of CYP2C9 affects warfarin, phenytoin and sulphonylurea drug dose requirements and has been associated with an increased risk of bleedingcomplications during warfarin treatment (van der Weide *et al.*, 2001, Higashi *et al.*, 2002, Kirchheiner *et al.*, 2005). The most common allelic variants with reduced catalytic activity are CYP2C9*2 and CYP2C9*3, and they have allele frequencies of 11% and 7%, respectively, in Caucasians (Kirchheiner and Brockmaller, 2005).

CYP2C19 metabolises proton pump inhibitors, some antidepressants, diazepam, proguanil and propranolol (Desta *et al.*, 2002). Omeprazole has been used as a probe inhibitor of CYP2C*19 both *in vitro* and *in vivo* (Ko *et al.*, 1997, Desta *et al.*, 2002). Other drugs, such as fluoxetine, fluvoxamine, ticlopidine and isoniazid, can also inhibit the metabolism of CYP2C19 substrate drugs (Desta *et al.*, 2002). Rifampicin and artemisinin have been identified as inducers of CYP2C19 (Desta *et al.*, 2002). Approximately 3-5% of Caucasians and up to 20% of Asian populations are poor metabolisers of CYP2C19 substrates (Desta *et al.*, 2002) CYP2C19 genotype has been shown to affect the efficacy of proton pump inhibitor reatments (Klotz *et al.*, 2004).

CYP2D6
CYP2D6 is the only functionally active isoenzyme in the CYP2D subfamily in humans. Although it constitutes only 2-5% of total CYP content in the liver, it is responsible for up to 25% of the metabolism of known drugs (Zanger and Eichelbaum, 2000, Ingelman-Sundberg, 2005). At lower levels, CYP2D6 is expressed in extrahepatic tissues, including the gastrointestinal tract and brain (Zanger *et al.*, 2004). CYP2D6 is polymorphically expressed, with four existing phenotypes that define the rate of drug metabolism by CYP2D6; poor metabolisers (PM), who lack the functional enzyme, intermediate metabolisers (IM), who have at least one partially deficient allele, extensive metabolisers (EM), who have two normal alleles, and ultrarapid metabolisers (UM), who have multiple gene copies. Up to 10% of Caucasians are poor metabolisers of CYP2D6 (Zanger *et al.*, 2004). CYP2D6 substrates include many tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), neuroleptics, beta-blockers and opiates (Zanger *et al.*, 2004). Quinidine, paroxetine, fluoxetine, fluvoxamine, moclobemide, flecainide and terbinafi

ne are potent inhibitors of CYP2D6 (Abdel-Rahman *et al.*, 1999, Zanger *et al.*, 2004). In contrast to all other CYP enzymes involved in drug metabolism, CYP2D6 is not known to be inducible (Ingelman-Sundberg, 2005).

CYP2E1

CYP2E1 accounts for about 14-17% of hepatic CYP content (Rowland-Yeo *et al.*, 2004, Klein *et al.*, 2006). This isoenzyme has mainly toxicological relevance, since it can bioactivate many compounds to carcinogens and reactive metabolites (Raycy and Carpenter, 2000). It takes part in the metabolism of ethanol, disulfiram, paracetamol, anaesthetics such as halothane, enflurane, isoflurane and sevoflurane, and chlorzoxazone, which serves as a probe of CYP2E1 activity (Raycy and Carpenter, 2000, Anzenbacher and Anzenbacherova, 2001). Ethanol and isoniazid are known inducers of CYP2E1 (Raycy and Carpenter, 2000). Disulfiram is reduced by CYP2E1 to diethyldithiocarbamate (DDC) (Raycy and Carpenter, 2000), which is used as an *in vitro* inhibitor of CYP2E1 (Eagling *et al.*, 1998).

3. CYP3A subfamily

CYP3A enzymes are the most important oxidative enzymes in human drug metabolism. They have been estimated to participate in the metabolism of 40-50% of all drugs (Pelkonen *et al.*, 1998, Wrighton *et al.*, 2000, Ingelman-Sundberg., 2004). The isoforms of CYP3A in humans include CYP3A4, CYP3A5, CYP3A7 and CYP3A43.

CYP3A4 is the most abundant CYP enzyme in both the small intestinal mucosa and the liver, where it accounts for almost 30% of total CYP content (Shimada *et al.*, 1994, Wrighton and Thummel, 2000). Its substrates include the calcium-channel blockers nifedipine, felodipine, diltiazem and verapamil, the HMG-CoA reductase inhibitors atorvastatin, lovastatin and simvastatin, the HIV protease inhibitors, the PDE5 inhibitors such as sildenafil, and the benzodiazepines alprazolam, midazolam and triazolam (Dresser *et al.*, 2000). Hepatic and intestinal CYP3A4 can be induced by several drugs, such as carbamazepine, phenytoin and rifampicin, and St. John's wort (*Hypericum perforatum*) (Backman *et al.*, 1996a, Backman *et al.*, 1996b, Durr *et al.*, 2000, Wrighton and Thummel, 2000). There are many known potent inhibitors of CYP3A4, including the azole antifungals ketoconazole, itraconazole and voriconazole, the macrolide antibiotics clarithromycin, erythromycin and troleandomycin, the calcium-channel blockers diltiazem and verapamil, the HIV protease inhibitors and grapefruit juice (Dresser *et al.*, 2000, Wrighton and Thummel, 2000; Theuretzbacher *et al.*, 2006). Although the activity of CYP3A4 varies greatly, its population distribution is unimodal and genetic polymorphisms do not appear to explain the interindividual variation (Wilkinson, 2005). CYP3A5 is significantly expressed in only about 10-20% of Caucasian livers (Lamba *et al.*, 2002). It is also found in extrahepatic tissues and is the dominant CYP3A form in the human kidney (Wrighton and Thummel, 2000). In individuals expressing CYP3A5, the contribution relative to the total hepatic CYP3A seems to range from 17% to 50% (Daly, 2006). CYP3A7 is present primarily in fetal tissues, representing about 50% of the total CYP in fetal liver (Wrighton and Thummel, 2000). It is also expressed in some adult livers (Daly, 2003). The substrate

specificities of CYP3A5 and CYP3A7 are, in general, similar to that of CYP3A4 (Wrighton and Thummel, 2000). Variable expression of CYP3A5 and CYP3A7 may account in part for the variation in the metabolism of CYP3A4 substrates (Daly, 2003). CYP3A4 is expressed in relatively high levels in the prostate and testes, but its expression in the liver is low. The functional role and substrate specificity of CYP3A43 are currently unknown (Daly, 2006).

3. Induction and inhibition of CYP enzymes

Induction has been suggested to be an adaptive process in which prolonged exposure to drugs or other chemicals causes an up-regulation in the amount of enzymes that are capable of metabolising the inducing agent. Since induction affects the rate of protein synthesis (or degradation), a steady state with respect to induction is generally reached in two to three weeks. The disappearance of the induction effect (wash-out period) after discontinuation of the inducing agent can also take several weeks (Wilkinson, 2005).

Induction of drug-metabolising enzymes may increase the elimination and reduce the bioavailability of the substrate drug, and correspondingly, decrease the drug's plasma concentration. In contrast, for drugs that are metabolised to active or reactive metabolites, induction can lead to enhanced drug effects or toxicity (Pelkonen *et al.*, 1998).

Inhibition of drug-metabolising enzymes can lead to increased plasma concentration of the substrate drug, and thus, exaggerated and prolonged pharmacological effects. This increases the likelihood of adverse effects and drug toxicity, especially with drugs that are extensively metabolised and have a narrow therapeutic index, unless appropriate dose reductions are made. In the case of prodrugs requiring metabolic activation, inhibition can reduce the clinical efficacy of the substrate drug. Contrary to induction, inhibition may occur immediately after one or two doses of the inhibitor (Lin and Lu, 1998).

3.1 Mechanisms of induction

Induction of drug-metabolising enzymes is mainly mediated by intracellular nuclear receptors. These include the pregnane X receptor (PXR), the constitutive androstane receptor (CAR) and the aryl hydrocarbon receptor (AhR) (Handschin and Meyer, 2003).

PXR and CAR mediate the induction of CYP2 and CYP3 enzymes, but phase II conjugative enzymes and drug transporters can also be induced (Honkakoski *et al.*, 2003). The mechanism of induction involves binding of the inducing agent to PXR or CAR. The complex then forms a heterodimer with the retinoid X receptor (RXR), which in turn binds to DNA-responsive element and enhances the transcription of the target gene (Willson and Kliewer, 2002). PXR and CAR seem to have flexible and overlapping binding specificities, and they can activate each other's target genes. Human PXR is activated by a wide range of structurally diverse chemicals, such as rifampicin, ritonavir and hyperforin, and phenobarbital has been shown to activate human CAR (Honkakoski *et al.*, 2003). Both PXR and CAR are abundantly expressed in the liver and intestine, with little expression appearing in other tissues.

Polycyclic aromatic hydrocarbon compounds found in tobacco smoke and grilled food induced drug-metabolising enzymes by binding to the AhR. This complex, together with another protein, AhR nuclear translocator (Arnt), increases enzyme expression by binding to the target gene's responsive element. This mechanism activates mainly CYP1A1 and CYP1A2, but the concentrations of glutathione S-transferase and UDPglucuronosyltransferase enzymes are also increased (Fuhr, 2000). Other known nuclear receptors, including the farnesoid X receptor (FXR) and the peroxisome proliferator activated receptor (PPAR), have also been shown to take part in regulating the expression of drug disposition genes (Dixit *et al.*, 2005). Contrary to nuclear receptor-mediated induction, ethanol can induce CYP2E1 by stabilisation of the enzyme, which results in accumulation of CYP2E1 (Fuhr, 2000).

3.2 Mechanisms of CYP inhibition

A drug can inhibit the activity of the CYP that metabolises the drug itself, but inhibition of other CYPs that play no role in the metabolism of the inhibitor itself also occur. The mechanism of CYP inhibition divides the inhibitors into reversible and irreversible (and quasi-irreversible) categories (Lin *et al.*, 1998).

1. Reversible inhibition

Reversible CYP inhibition is thought to be the most common mechanism causing pharmacokinetic drug–drug interactions. This type of inhibition is dose-dependent, and when the inhibitor is eliminated from the body, the normal metabolic function of the inhibited enzyme continues. Reversible inhibition can be further divided into competitive (prevalent), noncompetitive and uncompetitive types. The affinity with which the inhibitor binds to the enzyme is described by the inhibition constant (Ki), used to describe the inhibitory potency of a competitive inhibitor. The subtypes of reversible inhibition can be recognised according to the changes they produce in the observed kinetic constants of affected substrates *in vitro*, and Ki can be determined with graphical plotting methods or nonlinear regression models (Hollenberg, 2002).

In competitive inhibition, the substrate and inhibitor compete with each other for the active site of the enzyme. The inhibitor can share structural similarity with the substrates of the inhibited CYP, but this may not be apparent. Binding of the inhibitor to the active site of the enzyme prevents the binding and biotransformation of the substrate. In non-competitive inhibition, the inhibitor binds to the enzyme at a site distinct from the active site. The binding of the substrate to the enzyme active site is not affected, but the catalytic function of the enzyme-inhibitor-complex is not normal (Hollenberg, 2002). In uncompetitive inhibition, the inhibitor binds only to the enzyme-substrate complex, that is, binding of the substrate to the enzyme must precede binding of the inhibitor (Hollenberg, 2002).

2. Irreversible inhibition

Irreversible and quasi-irreversible inhibition require metabolic activation of the inhibitor by the affected CYP enzyme before forming an enzyme-inhibitor complex (Thummel *et al.*, 1998). Irreversible inhibitors are commonly called "catalysis-dependent", "mechanism-based" or "suicide" inhibitors. Covalent binding

of the activated inhibitor to the enzyme leads to irreversible inactivation of the enzyme (Kent *et al.*, 2001). The formed covalent bonds between an irreversible inhibitor and enzyme cannot be broken to regenerate the enzymatic activity. In vivo, this sort of inhibition may start more slowly than reversible inhibition, but the final effect is usually more profound and is reversed only by synthesis of new CYPs (Kent *et al.*, 2001).

4. Clinical outcomes and management of mechanism-based inhibition of cytochrome P450 3A4

Mechanism-based inhibition of cytochrome P450 (CYP) 3A4 is characterized by NADPH-, time-, and concentration-dependent enzyme inactivation, occurring when some drugs are converted by CYPs to reactive metabolites. Such inhibition of CYP3A4 can be due to the chemical modification of the heme, the protein, or both as a result of covalent binding of modified heme to the protein. The inactivation of CYP3A4 by drugs has important clinical significance as it metabolizes approximately 60% of therapeutic drugs, and its inhibition frequently causes unfavorable drug–drug interactions and toxicity. The clinical outcomes due to CYP3A4 inactivation depend on many factors associated with the enzyme, drugs, and patients. Clinical professionals should adopt proper approaches when using drugs that are mechanism - based CYP3A4 inhibitors. These include early identification of drugs behaving as CYP3A4 inactivators, rational use of such drugs (eg, safe drug combination regimen, dose adjustment, or discontinuation of therapy when toxic drug interactions occur), therapeutic drug monitoring, and predicting the risks for potential drug–drug interactions. A good understanding of CYP3A4 inactivation and proper clinical management are needed by clinical professionals when these drugs are used.

The human cytochrome P450 (CYP) 3A subfamily, includes CYP3A4, 3A5, 3A7 (Nelson *et al* 1996), and 3A43 (Domanski *et al* 2001). CYP3A4 is most abundant in the human liver (~ 40%) and metabolizes more than 50% of clinically used drugs (Shimada *et al* 1994; Rendic and Di Carlo 1997). Significant interindividual variability in the expression and activity of CYP3A4 has also been observed (Shimada *et al.*, 1994; Thummel *et al* 1994; von Richter *et al.*, 2004; Watanabe *et al.*, 2004). Such a substantial variability is considered to be a result of environmental, physiological, and genetic factors (Gibson *et al.*, 2002). CYP3A4 is known to metabolize a large variety of compounds varying in molecular weight from lidocaine (Mr = 234) to cyclosporine (Mr = 1203) (Guengerich 1999; Rendic 2002). It is also subjected to reversible and mechanism-based inhibition by a number of drugs. The latter involves the inactivation of the enzyme via the formation of metabolic intermediates (MIs) that bind tightly and irreversibly to the enzyme (Silverman 1998; Kent *et al.*, 2001).Mechanism-based inactivation of CYP3A4 by drugs can be due to the chemical modification of the heme, the protein, or both as a result of covalent binding of modified heme to the protein (Osawa and Pohl, 1989; Ortiz de Montellano and Correia, 1995; Silverman, 1998).

A mechanism-based inhibition of CYP3A4 is characterized by NADPH-, timeand concentration-dependent enzyme inactivation and substrate protection (Ito *et al* 1998b; Silverman, 1998). Human liver microsomes, cDNAexpressed enzyme, and hepatocytes are commonly used in in-vitro models for the investigation of

mechanism-based inhibition of CYP3A4 (Silverman, 1998). Important kinetic parameters for mechanism-based inhibition such as KI (the concentration required for half-maximum inactivation), $k$inact (the rate constant of maximum inactivation at saturation, analogous to Vmax), and partition ratio (Rmax, ratio of moles of substrate activation per mole of enzyme inactivation) can be determined using in vitro models. However, in vivo animal and human studies are usually needed to explore the clinical importance of CYP3A4 inactivation.

5. Drug transporters

It has become increasingly evident that active drug transport systems influence the pharmacokinetics of many drugs by controlling their movement into and out of cells (Giacomini and Sugiyama, 2006). Transporters work in concert with drug-metabolising enzymes, and it is thus oft en appropriate to consider together the impact of CYP-mediated drug metabolism and transporter-mediated drug effl ux and uptake when making assessments of drug pharmacokinetics (Ho and Kim, 2005). Drug interactions can occur when induction or inhibition of drug transporters alter e.g. intestinal absorption, proximal renal-tubular excretion, biliary excretion or penetration across the blood-brain barrier of substrate drugs (Giacomini and Sugiyama, 2006). The best-known drug transport systems that play a role in drug interactions are the P-glycoprotein (MDR1, multidrug resistance protein 1) and human organic aniontransporting polypeptides (OATPs) (DuBuske., 2005).

The P-glycoprotein is a transmembrane protein that operates as an efflux pump to export drugs out of cells. It facilitates excretion of substances into urine, bile and intestinal lumen (i.e. reduced absorption) and prevents excess accumulation in the brain (Fromm, 2003). There is overlapping specificity between CYP3A4 and P-glycoprotein substrates and inhibitors (Ayrton and Morgan, 2001). Substrate drugs for P-glycoprotein include anticancer drugs, HIV protease inhibitors, steroids, digoxin, quinidine, cyclosporine and loperamide (Giacomini and Sugiyama, 2006). Quinidine, erythromycin, verapamil and itraconazole are known inhibitors of P-glycoprotein (DuBuske, 2005, Ho and Kim, 2005). P glycoprotein is inducible by, for example, rifampicin and St. John's wort (Fromm, 2003).

OATPs are a class of transmembrane proteins that are expressed in human intestine, liver, kidney and brain tissue (Ho and Kim, 2005). In contrast to the P-glycoprotein, OATPs act as drug uptake pumps, transporting drugs into cells. Substrates for members of the OATP subfamily include bile salts, hormones, HMG-CoA reductase inhibitors, digoxin and methotrexate (Ho and Kim, 2005; Shitara et al., 2005).

Many agents that affect P-glycoprotein function also affect OATP activity. Coordinate activity of both drug uptake and efflux transporters may determine the net absorption and subsequent elimination of a drug (Kim, 2003). In addition to drug-induced changes in P-glycoprotein and OATP activity, these transporters also exhibit genetic polymorphism (Ho and Kim, 2005, Konig et al., 2006).

OBJECTIVES

To investigate the effect of ketoconazole on the pharmacokinetics of a single oral dose of risperidone in healthy Thai male volunteers.

MATERIALS AND METHODS

1 Materials
1.1 Chemical Standards
Working standard risperidone was purchased from Synfine Research Inc., Canada, Lot. No. A-1208-102, purity 99.8%. Working standard 9-hydroxyrisperidone was also purchased from Synfine Research Inc., Canada, Lot. No. S-1203-194A1, purity 98.5%, carbamazepine was purchased from Karindo, Italy, Lot. No. CB-14/06, purity 99.92%.

1.2 Chemicals and reagents
Acetonitrile HPLC grade and diethyl ether were purchased from Labscan Co., Ltd., Ireland. Sodium hydroxide and Acetic acid were obtained from Merck Co., Ltd. Analytical grade methanol was purchased from J.T.Baker, USA. Ammonium acetate was purchased from Ajax Finechem, Australia.

1.3 Equipments
Liquid chromatography tandem mass spectrometry consisted of Agilent 1200 pump, autosampler and column oven (Agilent Technologies, UK). Ion-trap model mass spectrometer (LC-MSD trap XCT, Agilent Technologies, UK) was connected to liquid chromatography system. The column (Alltima HP, Germany) was reverse phase C18, particle size 5 micron, diameter 2.1 mm, length 150 mm. A guard-pak precolumn module was used to obviate rapid column degeneration.

1.4 Computerized software
WinNonlin® version 1.1 (Pharsight, USA) was used to compute the pharmacokinetic data from plasma concentration of risperidone and 9-hydroxyrisperidone.

2 Method
2.1 Subjects
The subjects of this study were physically and mentally normal Thai male volunteers, aged 18-45 years old with BMI 18-25 kg/m^2. All were in good health on the basis of medical history and physical examination, routine blood test including complete blood count (CBC), white blood cell count, blood urea nitrogen (BUN), creatinine, SGOT (AST), SGPT (ALT), alkaline phosphatase (ALP), bilirubin, total protein and albumin. The subjects who have abnormal hematological, liver or kidney functions were excluded from the study. Subjects with known contraindication or hypersensitivity to the antipsychotic drug as well as those with known history of alcoholism or drug abuse were excluded. Drinking of alcoholic beverages, coffee and tea were not allowed at least 1 month prior to and during the entire period of the study. After complete explanation of the study, written informed consent was obtained from all subjects. The study protocol was approved by the Ethics Committee, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

2.2 Selection Criteria for Subjects

Inclusion criteria:

- Healthy Thai male volunteers with the ages range from 18 to 45 years and body mass index between 18 to 25 kg/m^2.
- Normal physical examination and clinical laboratory test.
- No history of illness described in exclusion criteria.
- No history of medication taken within 14 days before study.
- Willing to give written informed consent.

Exclusion criteria:

- Allergic or having adverse drug reaction to ketoconazole or risperidone or other inactive ingredient of risperidone product.
- History of gastrointestinal tract diseases, hepatic diseases, renal diseases, allergic disease, cardiovascular diseases, seizure or other disease states that affect to bioavailability of drug as shown in the following criteria:

    Hypertension: Sitting diastolic blood pressure more than 95 mmHg.

    Hepatic diseases: Raised hepatic enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), more than twice of the upper limit of normal range.

    Renal diseases: Serum creatinine more than 2.0 mg/dL.

    Gastrointestinal diseases: Signs and symptoms that interfere with drug absorption.

    Heart diseases: History of congestive heart failure, pericarditis, aortic stenosis, myocardial infraction, angina pectoris, cardiac arrhythmia or other heart disease.

- History of medication taken within 14 days before the study and during the study period.
- Clinically significant laboratory abnormalities.
- Habit smoker or history of routine smoking within 2 years before this study.
- History of alcohol and narcotic addiction.
- Join other experiment within 30 days before this study.
- Refuse to finish the study.

2.3 Subject Monitoring

Blood pressure, pulse rate and body temperature were monitored every 2 hours after drug administration. Subjects were asked for unusual symptoms periodically. All serious symptoms were immediately managed by doctors and recorded in the case report forms.

2.4 Protocol

The study was an open-label, randomized, two-phase crossover design with a 2-week washout period. The protocol was approved by the Ethics Committee, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

Study design
  Phase 1: Subjects receive a single oral dose of risperidone (2 mg) alone.
  In the morning after an overnight fasting, each subject received a single oral dose of 2 mg of risperidone. The drug was administered with a glass of water (240 ml) under supervision. No food was taken at least 2 hours after ingestion of the drug.

A catheter was inserted into a forearm vein for the collection of blood samples , and was maintained patent using 1 ml of a dilute heparin solution (100 unit/ml) after each sample. Venous blood samples (5 ml) were collected in heparinized tubes at the following time interval: pre-dose (before drug administration), 0.15, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72 and 96 hours after drug administration. Within 30 minutes after collection, all blood samples were centrifuged at 3,000 rpm for 10 minutes under the temperature of 4 °C. Collected plasma sample were stored at -70 °C until analysis.

Phase 2: Subjects received a single oral dose of risperidone after pretreatment with ketoconazole.
  After 2 weeks of being free from the drug, the subjects received ketoconazole capsules at an oral dose of 200 mg once daily after breakfast for 3 days prior to single oral dose of risperidone administration. In day 3 after an overnight fasting, all subjects took 2 mg of risperidone orally after ketoconazole administration for 0.5 hour. Venous blood samples were collected at the time interval before and after risperidone administration as previously described in phase 1.

2.5 Analysis of Risperidone and 9-hydroxyrisperidone in plasma
  A LC-MS/MS method was modified from Remmeries *et al* (2003) and validated to determine risperidone and 9-hydroxyrisperidone simultaneously, as described below.

2.6 Sample preparation
  20 µl of internal standard solution (5,000 ng/ml carbamazepine in methanol) was added to 1 ml of plasma sample. Then 150 µl of 80% w/v of sodium hydroxide was added and mixed for 10 seconds. Diethyl ether 6 ml was immediately added into the alkalinized-plasma sample and reciprocated for 2 minutes. The mixture was centrifuge at 3000 rpm for 10 minutes at temperature of 0°C. Ether layer was collected into another test tube and allowed to evaporate under room temperature. The residues were reconstituted with 150 µl of mobile phase before an aliquot of 40 µl was injected to the LC-MS/MS system.

2.7 Chromatographic system
Column	: Alltima HP (C18), stainless steel column, 2.1 (i.d.) x 150 mm, 5 µm of dimethyloctadecylsilyl bond silica.
Mobile phase	: 50 mM ammonium acetate buffer pH 5.5:acetonitrile (65 : 35)
Flow rate	: 0.3 ml/min.
Temperature	: 30 °C

Retention time : 9-hydroxyrisperidone   3.3 min.
                  Risperidone   4.3 min.
                  Carbamazepine   7.2 min.

2.8 Mass spectrometric detection

All studied compounds were completely separated with isocratic mobile phase before being introduced to electrospray ionization chamber. Transitions of positive ions were detected in selected reaction monitoring (SRM) mode. In an analytical run time, mass spectrometric detections were separated into three segments.

2.9 Calibration samples

Quantitative amount of standard 9OHRIS and RIS were added into the pooled drug free plasma to produce a series of concentration of 0.3 (9OHRIS)/0.1(RIS), 0.5, 1, 5, 10, 20 and 30 ng/mL, respectively. All calibration samples were analyzed following the same procedure as describe earlier. The peak area ratios of each RIS and 9OHRIS to CBZ (internal standard) versus known concentration were fitted to straight line using linear regression analysis.

2.10 Quality control samples

Quality control samples (QCs) are samples with known concentration prepared by spiking drug-free biological fluid with analyzed-drug. Quantitative amount of standard 9-hydroxyrisperidone and risperidone were add to the pooled drug free plasma to produce the series of the concentration of 0.5, 5 and 20 ng/mL, respectively. All quality control samples were analyzed following the same procedure as described earlier. The estimated concentration of quality control samples were calculated using the peak area ratios of each RIS and 9OHRIS to CBZ.

2.11 Bioanalytical method validation

The LC-MS-MS method for the simultaneous determination of risperidone and 9-hydroxyrisperidone in human plasma was validated according to Guideline of the Office of Food and Drug Administration, Thailand, 2006 described as follow:

1. Specificity

Six sources of blank plasma samples were analyzed using the same procedure as described before. The study samples should be demonstrated that the interested peaks were not interfered from endogenous compounds, degradation products, or other drugs.

2. Lower limit of quantification (LLOQ)

Six determinations of the lowest concentration of calibration samples were analyzed. The reliable LLOQ should be established based on the relative standard deviation (%RSD) not greater than 20% and the % accuracy should be within 80 – 120%.

3. Range and Linearity

The quantitative relationship between concentration and response should be adequately characterized over the entire range of expected sample concentrations. Seven concentrations of calibration samples of the mixture of 9-hydroxyrisperidone and risperidone (0.3(9OHRIS) and 0.1(RIS)), 0.5, 1, 5, 10, 20, 30

ng/mL) were analyzed. The linear relationship between the peak area ratios of risperidone and 9-hydroxyrisperidone versus concentrations calculated using regression analysis. The coefficient of determination (r^2) should be more than 0.99. The 20% deviation of the LLOQ from nominal concentration and 15% deviation of other concentration of both risperidone and 9-hydroxyrisperidone should be met.

4. Accuracy

Accuracy is the degree to which the true value of the concentration of drug is estimated by the assay. Five replications of three concentrations of calibration samples of risperidone and 9-hydroxyrisperidone (0.5, 5 and 20 ng/ml) were analyzed for drug content. The term %accuracy was calculated from the percentage of the ratio between the estimated concentration using calibration samples and the theoretical concentration. The mean value of both compounds should be within the range of 85.0 % - 115.0%.

5. Within-run precision

Five replications of three concentrations of calibration samples of risperidone and 9-hydroxyrisperidone (0.5, 5 and 20 ng/ml) were analyzed within the same run. The %RSD of the estimated concentrations from five replications were calculated. Within-run precision in term of %RSD should be not more than 15% and not more than 20%RSD at the lowest concentration.

6. Between-run precision

Five replications of three concentrations of calibration samples of risperidone and 9-hydroxyrisperidone (0.5, 5 and 20 ng/ml) were analyzed on five difference runs. The %RSD of the estimated concentrations from five replications were calculated. Between-run precision in term of %RSD should be not more than 15% and not more than 20% RSD at the lowest concentration.

7. Recovery of extraction

Five replications of extraction recovery at low, medium and high concentration (0.5, 5 and 20 ng/ml) of quality control samples were analyzed. Recovery of extraction was calculated from the percentage of the ratios between the concentration of each extracted quality control sample and the concentration of un-extracted standard at the same concentration.

8. Stability

8.1 Freeze-thaw stability

Three replications of two concentrations of quality control samples at low and high concentrations (0.5 and 20 ng/ml) were stored at -20°C for 24 hours and allowed the frozen plasma to dissolve at room temperature (thaw). The thawed samples were refrozen for 24 hours under the same conditions. This procedure defined as one freeze-thaw cycle, and after three freeze-thaw cycles, samples were analyzed. The % deviation of the mean estimated concentration from the zero time should be within ± 15%.

8.2 Short-term stability

Three replications of two concentrations of quality control samples at low and high concentrations (0.5 and 20 ng/ml) were prepared and stored at -20°C for 24 hours. After that, the samples were thawed at room temperature and analyzed after being kept at 0, 6, 10 and 24 hours according to the same procedure as described. The %deviation of the mean estimated concentration from the zero time should be within ± 15%.

8.3 Long-term stability

Three replications of two concentrations of quality control samples at low and high concentrations (0.5 and 20 ng/ml) were prepared and stored at -20°C for 0, 1, 2 and 3 months. After that, the samples were thawed at room temperature and analyzed according to the same procedure as described. The %deviation of the mean estimated concentration from the zero time should be within ± 15%.

8.4 Post-preparative stability (Auto-sampler stability)

In order to establish the auto-sampler stability of risperidone in human plasma matrix, two aliquots of low (0.5 ng/ml), and high (20 ng/ml) QC samples were stored in auto-sampler for 6, 12, 24, 48 and 72 h. Thereafter, samples were analyzed and concentrations were compared with concentration at zero time. The samples met the criteria of stability if the deviation was within ± 15%.

8.5 Stock solution stability

9OHRIS, RIS and CBZ were prepared by dissolving suitable amount of each pure substance in methanol and kept in room temperature for, 6, 14, and 24 h. After that they were stored in the temperature of -20°C for 15 days, 1, 2, and 3 months. Stock solutions were diluted with the mobile phase to the concentration of 10 μg/ml and they were compared to that of freshly prepared solutions of the same concentration. The samples met the criteria of stability if the deviation was within ± 2%.

RESULTS

1. Subjects

Ten healthy Thai male volunteers were enrolled in the study with ranging in age from 23 to 44 years (33.3 ± 8.11 years), in weight from 55 to 76 kg (64.15 ± 5.88 kg), and in height from 155 to 178 cm. (165.75 ± 7.29cm.). No subject was withdrawn from the study. The demographic characteristics of all subjects are presented in Table 2. Medical history, physical, hematological and biochemical profiles were examined for the inclusion of healthy volunteers as indicated in Table 3.

Table 2 Demographic data of 10 healthy male volunteers enrolled in the study.

Subject No.	Age (yr)	Weight (kg)	Height (cm)	BMI (kg/m^2)
SR01	44	63	160	24.61
SR02	35	67	164	24.91
SR03	44	62.5	158.5	24.88
SR04	41	57	155	23.73
SR05	36	65	165	23.88
SR06	23	62	178	19.57
SR07	30	55	164	20.45
SR08	25	66	168	23.38
SR09	32	76	176	24.54
SR10	23	68	169	23.81
Mean	**33.3**	**64.15**	**165.75**	**23.37**
S.D.	**8.11**	**5.88**	**7.29**	**1.86**

Table 3 Laboratory data of 10 healthy volunteers enrolled in the study

Subject No.	Hb (g %)	Hct (%)	WBC (cell/mm^3)	Platelets	FBS (mg%)	BUN (mg/dl)	Cr (mg/dl)	SGOT (u/l)	SGPT (u/l)	ALP (u/l)	TB (mg/dl)	ALB (mg/dl)	TP (mg/dl)
SR01	12.7	38	5700	Adequate	80	15	1.06	30	28	91	0.8	5.1	7
SR02	13.7	41	5800	Adequate	83	12	1.09	29	34	82	0.7	4.7	6.9
SR03	13	39	6800	Adequate	79	16	0.9	24	27	113	0.7	5.1	7.1
SR04	14	43	5000	Adequate	86	14	1.09	29	33	101	0.77	5.2	7.2
SR05	14.3	43	4700	Adequate	80	13	1.14	14	24	70	0.9	5	7.2
SR06	13	39	7100	Adequate	78	6	1.1	10	17	12.7	0.96	5.26	7.5
SR07	14	42	5700	Adequate	70	17	1.1	28	27	78	0.99	5.3	8.1
SR08	15.5	45	10900	Adequate	73	7	1.07	24	24	90	1	5.1	7.4
SR09	12.1	37	6800	Adequate	73	9	1.1	12	20	85	0.75	5	7.3
SR10	13	61	7500	Adequate	86	11	1	28	37	105	0.8	5.1	6.8
Mean	**13.53**	**42.8**	**6600**	**Adequate**	**78.8**	**12**	**1.06**	**22.8**	**27.1**	**82.77**	**0.84**	**5.09**	**7.25**
S.D.	**0.97**	**6.87**	**1765**	**Adequate**	**5.47**	**3.74**	**0.07**	**7.67**	**6.26**	**27.81**	**0.12**	**0.117**	**0.37**

2. Mass spectrometric detection

Due to the highest efficient of ionization and fragmentation of the interested compounds, the m/z transition were selected for their detection. Figure 4-6.showed the product ions of 9OHRIS, RIS and CBZ. With positive ion mode and electrospray ionization (ESI) conditions, 9OHRIS gave M+H$^+$ at m/z 427.4 as the precursor ion. This ion was selected to generate one major product ion at m/z 207.4 representing the cleavage of the ethyl-piperidinyl bond. RIS gave M+H$^+$ at m/z 411.4 as the precursor ion. This ion was selected to generate one major product ion at m/z 191.4 representing the cleavage of the ethyl-piperidinyl bond. Internal standard, CBZ gave M+H$^+$ at m/z 237.3 as precursor ion. This ion was selected to generate one major product ion at m/z 194.0 representing the cleavage of the $CONH_2$ group from the ring.
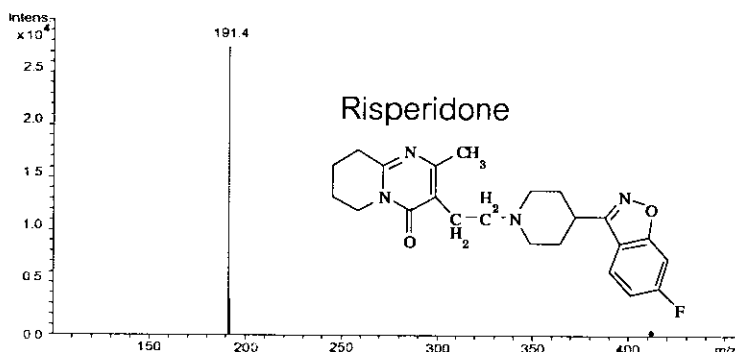

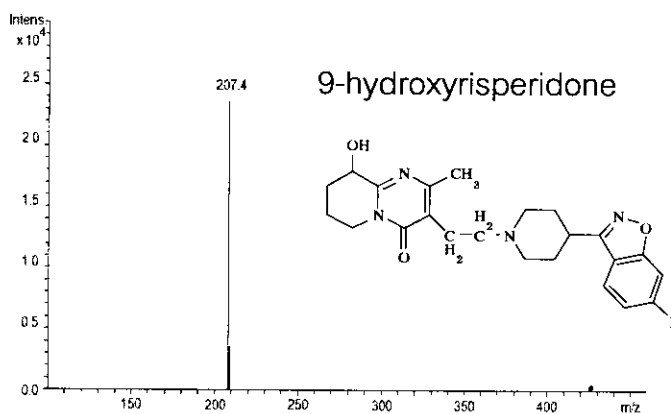**Figure 4** Electrospray ionization mass spectrum of product ion of risperidone

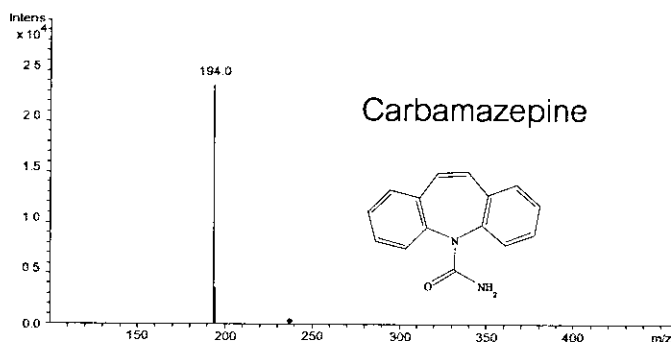**Figure 5** Electrospray ionization mass spectrum of product ion of 9-hydroxyrisperidone

Figure 6 Electrospray ionization mass spectrum of product ion of carbamazepine

50

3. Sample preparation

Sample preparation was achieved by adjusting the sample pH to more than 10, thus converting the analytes (pKa ~ 8) into their neutral forms, and extracting them into an organic solvent. Pure solvents and the mixture of solvents were investigated based on their polarity for sample extraction. In this step, the spiked plasma samples at concentration of 5ng/ml were extracted with each solvent. The extraction efficiency of each solvent was then evaluated by comparing the extraction recovery with un-extracted standard at the same concentration.Results indicated that RIS and CBZ could be extracted into all solvent systems with more than 80% extraction recovery. However, for 9OHRIS, a polar solvent was required to obtain its reasonable recovery. It was found that the highest recovery of 9OHRIS was achived when the sample was alkalinized with 80% w/v NaOH before extraction with ether.

4. Method Validation
1. Specificity

The aim of specificity test was to ensure the authenticity of the results for the sample analysis. Six blank samples of pooled plasma from six different donors were analyzed to ensure that no endogenous compounds or other impurities interfered with the assay. Figure 7 to Figure 9 demonstrate the specificity results with chromatograms of pooled plasma blank, and the peak response of 9OHRIS, RIS and CBZ at LLOQ, respectively. 9OHRIS, RIS and CBZ were separated with the C18-chromatographic column and provided the retention times of 3.3, 4.3 and 7.2 min, respectively. All interested peaks were completely separated with isocratic elution mobile phase. No interferences of endogenous substances were observed.
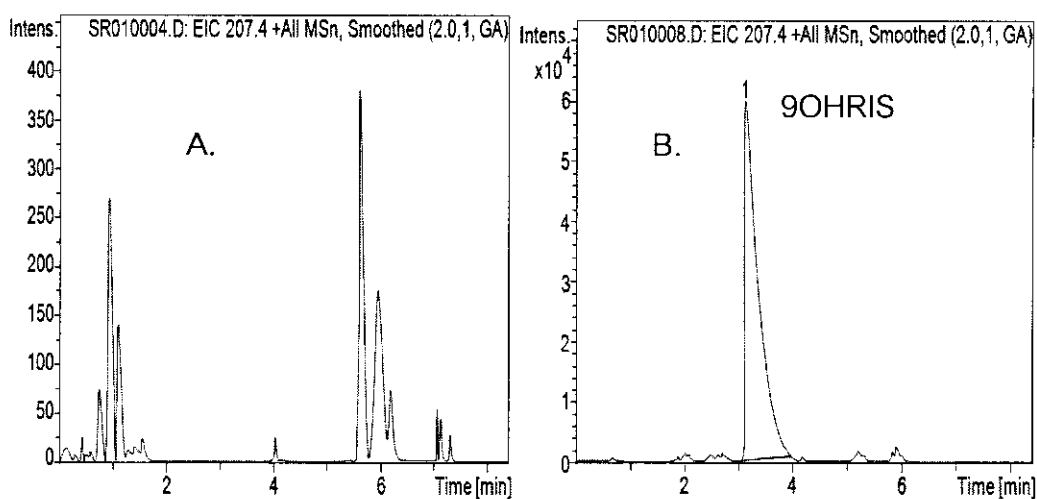

Figure 7 Chromatograms of blank plasma (A), and 9-hydroxyrisperidone (9OHRIS) (B) at LLOQ concentration (0.3 ng/ml).
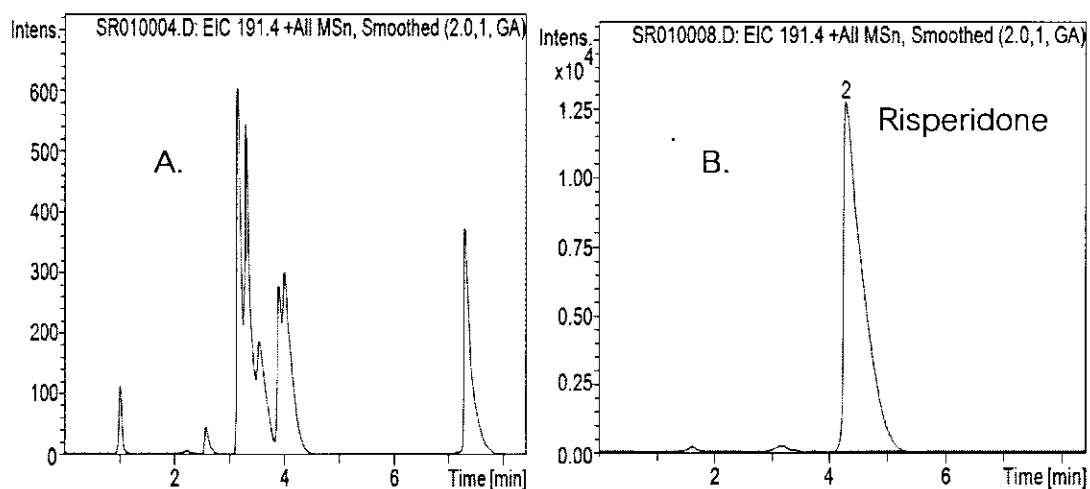
Figure 8 Chromatograms of blank plasma (A) and risperidone (B) at LLOQ concentration (0.1 ng/ml).
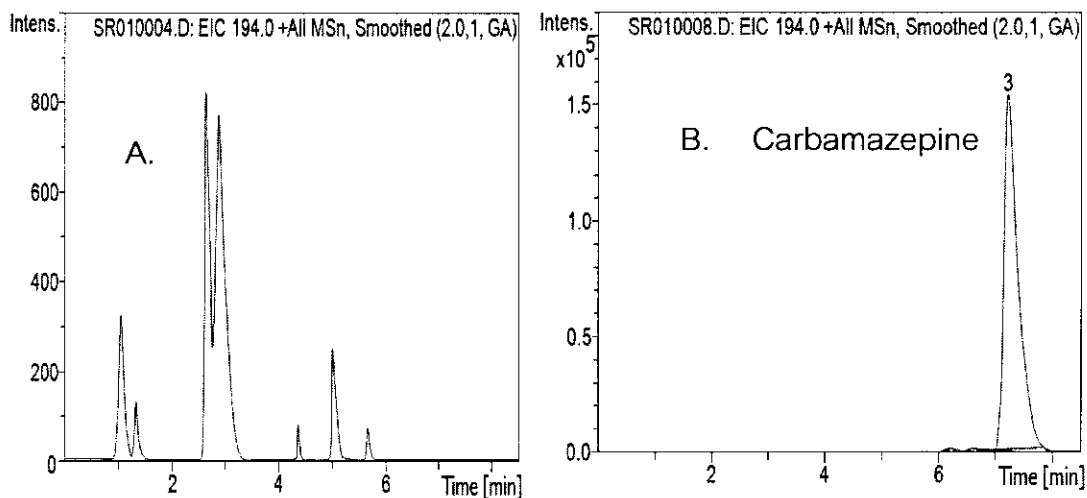

Figure 9 Chromatograms of blank plasma (A) and carbamazepine (B) 100 ng/ml.

2. Calibration curve and linearity

Linearity of 9OHRIS and RIS were examined by analyzing calibration curves containing seven standard concentrations of 9OHRIS (0.3, 0.5, 1, 5, 10, 20, and 30 ng/mL) and RIS (0.1, 0.5, 1, 5, 10, 20, and 30 ng/ml). As presented in Figure 17 the calibration curves of 9OHRIS and RIS showed linear response over the range of concentration used in assay procedure with the coefficient of determination (r^2) greater than 0.99. The equations for mean (n = 5) of five calibration curves for each analyte were: 9OHRIS; $y = 0.1631x + 0.0317$ ($r^2 = 0.9983$), RIS; $y = 0.3474x + 0.0654$, ($r^2 = 0.9981$), (Table 4 and 5).

Table 4 Calibration curve data of RIS in plasma.

No.	Theoretical Concentration (ng/ml)	Peak Ratio	Estimated Concentration (ng/ml)	%Difference
1	0.1	0.091044	0.09	-9.64
2	0.5	0.223016	0.47	-6.29
3	1	0.417956	1.03	2.97
4	5	1.916826	5.34	6.32
5	10	3.601027	10.18	1.74
6	20	6.662292	18.97	-5.41
7	30	10.680302	30.52	1.70

Table 5 Calibration curve data of 9OHRIS in plasma.

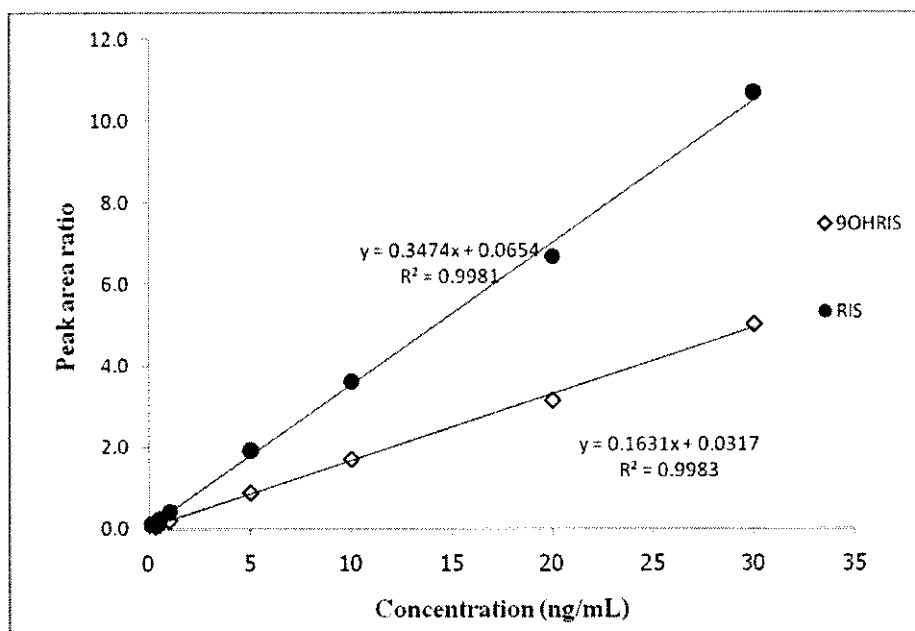| No.	Theoretical Concentration (ng/ml)	Peak Ratio	Estimated Concentration (ng/ml)	%Difference
1	0.3	0.07074	0.26	-17.63
2	0.5	0.10746	0.48	-4.21
3	1	0.20116	1.05	5.07
4	5	0.88920	5.27	5.05
5	10	1.70230	10.24	2.38
6	20	3.13585	19.02	-5.15
7	30	5.00824	30.48	1.58

Figure 10 Calibration curves of 9OHRIS and RIS in plasma.

3. LLOQ

The lower limit of detection of with acceptable accuracy (+20%) and precision (%RSD < 20%) of 9OHRIS was 0.3 ng/mL and that of RIS was 0.1 ng/mL as shown in figure 7 to figure 9 and Table 6 and 7. For the highest sensitivity, mass spectrometric detection was set to detect the *m/z* transitions with selected reaction monitoring (SRM) mode. Along the chromatographic run-time of 8.5 minutes, the mass spectrometric detection of *m/z* transition was separated into three segments according to chromatographic separation of each peak.

Table 6 LLOQ of RIS in plasma

	Peak area		Peak Ratio	Estimated Concentration (ng/ml)	%Accuracy
	RIS	**CAR**			
LLOQ1	380314	4544092	0.08369	0.1106	110.62
LLOQ2	378490	4923634	0.07687	0.0927	92.65
LLOQ3	300952	3702039	0.08129	0.1043	104.30
LLOQ4	353283	4099202	0.08618	0.1172	117.18
LLOQ5	370294	5019928	0.07376	0.0845	84.46
LLOQ6	378355	4672993	0.08097	0.1034	103.44
			Average	**0.1021**	
			%RSD	**11.65**	

Table 7 LLOQ of 9OHRIS in plasma.

	Peak area		Peak Ratio	Estimated Concentration (ng/ml)	%Accuracy
	9OHRIS	**CAR**			
LLOQ1	284362	4544092	0.06258	0.3092	103.06
LLOQ2	270317	4923634	0.05490	0.2541	84.69
LLOQ3	210238	3702039	0.05679	0.2676	89.20
LLOQ4	280354	4099202	0.06839	0.3509	116.97
LLOQ5	270283	5019928	0.05384	0.2465	82.15
LLOQ6	279192	4672993	0.05975	0.2888	96.28
			Average	**0.2862**	
			%RSD	**13.70**	

4. Accuracy and precision

The accuracy and precision of within- and between-run of analytical method for 9OHRIS and RIS in plasma were shown in Table 8.-11.The accuracy and precision was measured at each quality control samples level (QCs) ($n = 5$) within the analytical range at concentration of low (0.5 ng/ml), medium (5 ng/ml) and high (20 ng/ml) for both compounds. The accuracy of within- and between-run of these two compounds were within the range of 85.0 – 115.0%. For the precision, the %RSD of within- and between–run of both 9OHRIS and RIS were less than 15%.

Table 8 Within-run accuracy and precision data of risperidone (RIS}

Theoretical Concentration (ng/ml)	N	Average Concentration (ng/ml)	%RSD	%Accuracy
0.5	5	0.47 ± 0.03	6.28	93.29
5	5	5.18 ± 0.17	3.36	103.65
20	5	20.53 ± 0.69	3.38	102.65

Table 9 Within-run accuracy and precision data of 9OHRIS

Theoretical Concentration (ng/ml)	N	Average Concentration (ng/ml)	%RSD	%Accuracy
0.5	5	0.46 ± 0.04	8.20	92.26
5	5	5.05 ± 0.35	6.89	100.95
20	5	19.80 ± 0.60	3.04	99.00

Table 10 Between-run accuracy and precision data of RIS

Theoretical Concentration (ng/ml)	N	Average Concentration (ng/ml)	%RSD	%Accuracy
0.5	5	0.51 ± 0.03	5.78	102.17
5	5	5.05 ± 0.20	3.96	100.96
20	5	19.91 ± 0.77	3.87	99.57

Table 11 Between-run accuracy and precision data of 9OHRIS

Theoretical Concentration (ng/ml)	N	Average Concentration (ng/ml)	%RSD	%Accuracy
0.5	5	0.48 ± 0.03	7.14	96.79
5	5	5.00 ± 0.08	1.53	100.06
20	5	19.96 ± 0.53	2.67	99.81

5. Extraction recovery

Mean extraction recoveries of RIS and 9OHRIS at concentrations of 0.5, 5 and 20 ng/ml and CBZ at concentration at 100 ng/ml were shown in Table12.

Table 12 Extraction recovery of RIS, 9OHRIS, and CBZ in plasma.

Compound	Concentration (ng/ml)	N	Average %Recovery	%RSD
Risperidone	0.5	5	86.5 ± 7.8	4.38
	5	5	90.5 ± 11.6	5.65
	20	5	104.1 ± 9.5	9.02
9-hydroxyrisperidone	0.5	5	101.0 ± 4.4	8.96
	5	5	87.1 ± 4.9	12.80
	20	5	86.3 ± 7.8	9.10
Carbamazepine	100	5	96.4 ± 8.1	8.37

6. Stability

6.1 Freeze-thaw stability

The stability of 9OHRIS and RIS at the low (0.5 ng/ml) and high (20 ng/m) concentrations was studied after sample being subjected to three freeze-thaw cycles. It was both compounds were stable after three freeze–thaw cycles. From Table13-14, the percent deviation from zero time of 9OHRIS was -3.63 to 13.10%, and that of RIS was -9.91 to 2.59%. The coefficient of variation was less than 15% for all measured concentrations of both 9OHRIS and RIS. These results indicated that no tendency of their degradation after three freeze-thaw cycles was observed, as presented in Table 13-14.

6.2 Short-term stability

The short term stability of 9OHRIS and RIS were performed at room temperature. As shown in Table 15 and 16 QC sample at the concentrations of 0.5 and 20ng/ml were left for 6, 10, and 24 h. For 9OHRIS, the percent deviation from zero time was -10.81% to -6.00% and that of RIS was -10.69 to 4.70%. The percent deviation of both 9OHRIS and RIS were less than 15% for up to 24 h when kept at room temperature. Although the results indicated that plasma sample could be allow in room temperature for up to 24 hours, all plasma samples were thaw in room temperature for not more than 2 hour before extraction.

6.3 Long-term stability

The long-term stability of 9OHRIS and RIS in plasma was studied using QC samples at the concentration of 0.5 and 20 ng/mL. Triplicate-QCs were analyzed after being kept at -20°C for 1, 2 and 3 months. Due to the last plasma

58

sample was analyzed after drawn from subjects for 7 months, the 9-month -20°C-stored QCs (n=2) were analyzed to ensure the stability of 9OHRIS and RIS after long term storage. Both 9OHRIS and RIS plasma samples shown no tendency of degradation. The percent deviation of 9OHRIS was to -14.73% to 9.53% from the zero time and that for RIS was -4.45% to 10.85%, as shown in Table 17 and 18.

6.4 Post-preparison stability (Autosampler stability)

Stability of QC sample at concentration of 0.5 and 20 ng/ml stored in the autosampler over a period of 72 h was evaluated. QC sample were stored in autosampler until the time of injection at 12 h, 24 h, 48 h and 72 h. Table 19 and 20 shown that the percent deviation from zero time ranged from -2.20% to -11.04% for 9OHRIS at t = 0 to t = 24 h. At 48 hours, 9OHRIS concentration decreased with the percent deviation more than 15%. For RIS, percent deviation from zero time was 10.85% to -12.16% at the time t = 0 to t = 48. RIS seemed to be unstable at t = 72. The results shown in table 24 and 25 demonstrated that the samples should be kept in autosampler for not more than 24 hour. A 85-sample analytical batch consisted 1 blank-plasma sample, 68 duplicate-subject-plasma samples, 7 calibration-curve samples, 6 QCs samples and 3 recovery samples were analyzed within 15 hours which less than maximum limit that sample could be stored in autosampler.

7. Stock solution stability

Stability of stock solutions of 9OHRIS (0.1 µg/ml), RIS (0.1 µg/ml and CBZ (0.1 µg/ml) in methanol were investigated. The results presented in Table 21 shown that 9OHRIS and RIS and IS were stable in methanol for at least 3 months at -20˚C with the percent deviation from zero time not more than 2.0%. In this bioanalytical method validation indicated that the simultaneous determination of 9OHRIS and RIS in human plasma had been proven to be simple, specific, accurate and precise. The proposed validated method was applied for the assay of 9OHRIS and RIS in 10 healthy Thai adult male subjects who received 2 mg test and reference products of risperidone under the fast condition.

Table 13 Frezze-thaw stability data of RIS (n=3)

Theoretical Concentration (ng/ml)	t=0	1 cycle		2 cycles		3 cycles	
	Estimated Concentration (ng/ml)	Estimated Concentration (ng/ml)	%Difference	Estimated Concentration (ng/ml)	%Difference	Estimated Concentration (ng/ml)	%Difference
0.5	0.54 ± 0.04	0.52 ± 0.02	-3.46	0.55 ± 0.02	2.59	0.55 ± 0.06	1.91
20	20.02 ± 1.70	18.03 ± 0.77	-9.91	18.90 ± 0.70	-5.58	19.04 ± 0.88	-4.90

Table 14 Frezze-thaw stability data of 9OHRIS (n=3)

Theoretical Concentration (ng/ml)	t=0	1 cycle		2 cycles		3 cycles	
	Estimated Concentration (ng/ml)	Estimated Concentration (ng/ml)	%Difference	Estimated Concentration (ng/ml)	%Difference	Estimated Concentration (ng/ml)	%Difference
0.5	0.54 ± 0.04	0.52 ± 0.02	-3.46	0.55 ± 0.02	2.59	0.55 ± 0.06	1.91
20	20.02 ± 1.70	18.03 ± 0.77	-9.91	18.90 ± 0.70	-5.58	19.04 ± 0.88	-4.90

Table 15 Short term stability data of RIS (n=3)

Theoretical	t=0	6 hr	10 hr	24 hr

Table 15 Short term stability data of RIS (n=3)

Theoretical Concentration (ng/ml)	t=0	6 hr		10 hr		24 hr	
	Estimated Concentration (ng/ml)	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0
0.5	0.50 ± 0.04	0.50 ± 0.04	0.00	0.52 ± 0.03	4.58	0.52 ± 0.03	4.70
20	20.77 ± 0.74	19.02 ± 0.88	-8.41	19.20 ± 0.81	-7.55	18.55 ± 0.46	-10.69

Table 16 Short term stability data of 9OHRIS (n=3)

Theoretical Concentration (ng/ml)	t=0	6 hr		10 hr		24 hr	
	Estimated Concentration (ng/ml)	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0
0.5	0.50 ± 0.05	0.45 ± 0.02	-9.10	0.47 ± 0.05	-6.00	0.46 ± 0.05	-6.93
20	20.89 ± 1.25	19.01 ± 0.60	-8.99	19.64 ± 0.34	-6.00	18.63 ± 0.34	-10.81

Table 17 Long term stability data of RIS (n=3)

Theoretical Concentration (ng/ml)	t = 0	t = 1 month		t = 2 months		t = 3 months		t = 9 months (n=2)	
	Estimated Concentration (ng/ml)	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0
0.5	0.48 ± 0.03	0.57 ± 0.03	10.85	0.52 ± 0.03	1.35	0.52 ± 0.02	0.34	0.51 ± 0.03	-1.06
20	18.87 ± 0.25	18.78 ± 0.47	-1.45	18.55 ± 0.58	-2.66	19.79 ± 0.46	3.86	18.21 ± 0.25	-4.45

Table 18 Long term stability data of 9OHRIS (n=3)

Theoretical Concentration (ng/ml)	t = 0	t = 1 month		t = 2 months		t = 3 months		t = 9 months (n=2)	
	Estimated Concentration (ng/ml)	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0
0.5	0.54 ± 0.01	0.53 ± 0.03	-2.31	0.46 ± 0.03	-14.73	0.49 ± 0.05	-9.64	0.45 ± 0.04	-7.45
20	19.52 ± 1.03	21.38 ± 0.38	9.53	19.96 ± 0.91	2.26	20.04 ± 0.38	2.68	18.53 ± 0.26	0.75

Table 19 Autosampler stability data of RIS

Theoretical Concentration (ng/ml)	t=0	12 hour		24 hour		48 hour		72 hour	
	Estimated Concentration (ng/ml)	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviatio from t=0
0.5	0.52 ± 0.03	0.57 ± 0.04	10.85	0.49 ± 0.05	-4.20	0.48 ± 0.03	-6.91	0.45 ± 0.02	-12.16
20	19.06 ± 0.25	18.78 ± 0.91	-1.45	17.63 ± 1.05	-7.51	18.05 ± 1.13	-5.27	17.19 ± 0.79	-9.81

Table 20 Autosampler stability data of 9OHRIS

Theoretical Concentration (ng/ml)	t=0	12 hour		24 hour		48 hour		72 hour	
	Estimated Concentration (ng/ml)	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0
0.5	0.54 ± 0.01	0.50 ± 0.03	-8.04	0.48 ± 0.05	-11.04	0.46 ± 0.05	-15.52	0.42 ± 0.01	-22.37
20	19.52 ± 1.03	19.09 ± 1.00	-2.20	18.61 ± 1.52	-4.69	17.50 ± 0.91	-10.35	15.15 ± 1.04	-22.37

Table 21 Stock solution stability data of RIS, 9OHRIS and CBZ (n=3)

Compounds	Theoretical concentration (µg/ml)	t=0	6 hr		14 hr		24 hr	
		Estimated Concentration (mg/ml)	Estimated Concentration (mg/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0
RIS	10	9.96 ± 0.033	10.05 ± 0.004	0.90	10.09 ± 0.005	1.20	10.08 ± 0.019	1.20
9OHRIS	10	9.96 ± 0.002	10.10 ± 0.001	1.41	10.00 ± 0.028	0.40	10.06 ± 0.028	1.00
CBZ	10	10.07 ± 0.001	10.09 ± 0.009	0.20	9.98 ± 0.002	-0.89	10.00 ± 0.021	-0.10

Table 21 Stock solution stability data of 9OHRIS, RIS and CBZ (continue)

Compounds	Theoretical concentration (μg/ml)	15 days		1 month		2 months		3 months	
		Estimated Concentration (mg/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0	Estimated Concentration (ng/ml)	%deviation from t=0
RIS	10	9.95 ± 0.014	-0.10	10.01 ± 0.021	0.50	9.97 ± 0.032	0.10	9.96 ± 0.015	-0.02
OHRIS	10	10.03 ± 0.02	0.70	10.07 ± 0.011	1.10	9.98 ± 0.015	0.20	10.00 ± 0.045	0.40
CBZ	10	9.98 ± 0.020	-0.89	10.04 ± 0.003	-0.30	10.04 ± 0.019	-0.30	10.00 ± 0.034	-0.70

8. Pharmacokinetic Study

8.1 Pharmacokinetics of risperidone in phase 1 and phase 2

Risperidone, is atipical novel antipsychotic under investigation for treatment of schizophrenia is a disease of the brain. The present study of risperidone and ketoconazole in healthy ten male volunteers, A recent *in vivo* study has shown that risperidone is a substrate of P-glycoprotein and CYP3A4. The aim of this study was to confirm the effects of ketoconazole, a P-glycoprotein and CYP3A4 inhibitor on the pharmacokinetics of risperidone. When pretreatment with ketoconazole and fllowing coadministration of risperiodone may be lead to change the pharmacokinetic parameters of risperidone.

In the present study, an open, randomized, two phases crossover design with 2 weeks washout period was used. In phases 1, each subject in group 1 ingested a single dose of 2 mg of risperidone and in group 2, each subject ingested the same dose of risperidone after orally pretreatment with 200 mg of ketoconazole once daily for 3 days.

The results of this study had shown that the mean of C_{max}, plasma concentrations of risperidone could be detected in all 10 subjects. In the ketoconazole pretreated group, the mean C_{max} of risperidone was not significantly and slightly increased by 17.42% but the T_{max} of risperidone was lesser than in phase 1 by 12.5% but there was no significant different from the control group. The $T_{1/2}$ of risperidone was significantly increased by 32.8% and the AUC_{0-48} and AUC_{0-96} of risperidone were significantly increased by 53.7% and 55.37%), respectively and the Vd/f and the Cl/f of risperidone were significantly decreased by 23.24% and 39.82%, respectively (Table 24).

8.2 Pharmacokinetics of 9-hydroxyrisperidone in phase 1 and phase 2

The active metabolite of risperiedone was 9-hydroxyrisperidone. The result of pharmacokinetic parameters of 9-hydroxyrisperidone after the subjects receiving a single oral dose of risperidone compared with coadministration with ketoconazole have shown that the C_{max}, T_{max} and $T_{1/2}$ of 9-hydroxyrisperidone were not significantly decreased by 20.32%, 19.58%, and 22.48%, respectively but the Cl/f of 9-hydroxyrisperidone was significantly increased by 117.32%. These change led to corresponding decrease in the AUC_{0-48} and AUC_{0-96} of 9-hydroxyrisperidone which were significantly decreased by 50.05% and 49.95%, respectively but the Vd/f was not significantly increased by 47.24% (Table 24).

Table 22 Pharmacokinetic parameters of risperidone in each of ten subjects receiving a single oral dose of 2 mg ((Phase 1) compared with after pretreatment with ketoconazole 200 mg for 3 days (Phase 2).

Subject No.	C_{max} (ng/ml)		T_{max} (hr)		$T_{1/2}$ (hr)		AUC_{0-48} (ng/l/hr)		AUC_{0-96} (ng/l/hr)		Vd/f (l/kg)		Cl/f (l/hr/kg)	
	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2
1	19.55	16.64	1	1	4.01	6.28	62.50	97.89	63.15	103.04	0.1831	0.1759	0.0317	0.0194
2	23.02	14.99	1.5	1	11.78	18.93	113.39	181.98	114.36	196.02	0.2973	0.2787	0.0175	0.0102
3	15.32	23.46	0.5	0.75	1.44	2.945	22.39	58.65	23.87	60.05	0.1738	0.1415	0.0838	0.0333
4	13.64	13.75	1.5	1	3.12	4.14	34.68	63.19	37.37	66.36	0.2406	0.1801	0.0535	0.0301
5	8.27	16.53	0.75	0.75	1.92	1.61	24.48	28.718	24.76	29.20	0.2239	0.159	0.0808	0.0685
6	13.88	23.87	1	1	2.34	2.17	53.01	89.88	54.02	90.99	0.1249	0.0688	0.037	0.0220
7	21.99	23.48	0.75	0.75	6.17	6.45	117.92	143.73	123.28	149.09	0.1445	0.1248	0.0162	0.0134
8	7.92	18.54	1	1	3.42	2.53	30.97	64.34	32.35	66.14	0.3048	0.1104	0.0618	0.0302
9	18.74	14.56	1	0.75	7.36	10.77	107.59	149.70	111.57	157.88	0.1904	0.1969	0.0179	0.0127
10	23.73	29.15	1	0.75	7.15	8.85	145.21	216.59	147.12	218.34	0.1402	0.117	0.0136	0.0092
Mean	**16.60**	**19.5**	**1**	**0.875**	**4.87**	**6.46**	**71.21**	**109.47**	**73.190**	**113.71**	**0.2023**	**0.1553**	**0.0413**	**0.0249**
S.D.	**5.743**	**5.158**	**0.311**	**0.131**	**3.225**	**5.322**	**45.568**	**60.850**	**46.355**	**63.317**	**0.0632**	**0.0578**	**0.0269**	**0.0176**

Table 23 Pharmacokinetic parameters of 9-hydroxy risperidone in each of ten subjects receiving a single oral dose of 2 mg ((Phase 1) compared with after pretreatment with ketoconazole 200 mg for 3 days (Phase 2).

Subject No.	C_{max} (ng/ml)		T_{max} (hr)		$T_{1/2}$ (hr)		AUC_{0-48} (ng/l/hr)		AUC_{0-96} (ng/l/hr)		Vd/f (l/kg)		Cl/f (l/hr/kg)	
	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2
1	10.95	10.35	8	1	31.28	17.31	425.94	95.96	464.87	97.22	0.1942	0.5137	0.0206	0.0043
2	4.49	12.15	12	1.5	57.4	21.62	137.9	116.82	159.48	120.6	1.0386	0.5173	0.0125	0.0166
3	16.84	23.93	4	1	17.01	12.26	355.07	202.56	362.68	203.09	0.1353	0.1742	0.0055	0.0098
4	20.58	15.85	8	6	21.17	18.47	187.31	449.79	457.42	208.28	0.1336	0.256	0.0044	0.0096
5	18.61	23.78	1.5	0.75	13.88	8.13	296.65	242.93	322.48	243.2	0.1242	0.0965	0.0062	0.0082
6	28.7	19.43	1.5	4	15.74	20.14	762.5	512.36	767.9	530.67	0.0592	0.1095	0.0026	0.0038
7	17.91	10.34	6	12	22.36	25.95	492.73	287.97	507.54	305.88	0.1272	0.2448	0.0039	0.0065
8	15.86	9.05	6	2	10.11	18.43	365.23	193.39	365.26	196.85	0.0799	0.2702	0.0055	0.0102
9	18.98	6.08	5	12	35.34	24.48	542.35	194.97	576.59	203.4	0.1769	0.3474	0.0035	0.0098
10	15.43	3.17	8	8	24.22	25.85	409.22	82.18	426.57	98.37	0.1639	0.7583	0.0047	0.0203
Mean	16.835	13.413	6	4.825	24.851	19.264	397.49	237.893	441.079	220.756	0.2233	0.32879	0.00694	0.00991
S.D.	6.268	7.136	3.223	4.472	13.7965	5.77128	179.136	144.101	161.897	127.108	0.2893	0.2098	0.00551	0.00512

Table 24 Pharmacokinetic parameters (mean ± S.D.) and %change of risperidone and 9-hydroxyrisperidone in each of ten subjects receiving a single oral dose of risperidone 2 mg (Phase 1) compared with after pretreatment with ketoconazole 200 mg for 3 days (Phase 2).

Parameter	RISPERIDONE				9-HYDROXYRISPERIDONE			
	Phase 1	Phase 2	% change	P-value	Phase 1	Phase 2	% change	P-value
C_{max} (ng/ml)	16.60 ± 5.74	19.50 ± 5.15	17.42	NS	16.83 ± 6.27	13.42 ± 7.13	-20.321	NS
T_{max} (hr)	1 ± 0.31	0.875 ± 0.13	-12.5	NS	6 ± 3.22	4.825 ± 4.47	-19.583	NS
$T_{1/2}$ (hr)	4.87 ± 3.23	6.46 ± 5.32	32.8	$P < 0.05$	24.86 ± 13.8	19.27 ± 5.77	-22.48	NS
AUC_{0-48} (ng/l/hr)	71.21 ± 45.56	109.47 ± 60.85	53.7	$P < 0.05$	423.74 ± 163.46	211.65 ± 123.67	-50.052	$P < 0.05$
AUC_{0-96} (ng/l/hr)	73.19 ± 46.35	113.71 ± 63.31	55.37	$P < 0.05$	441.08 ± 171.63	220.76 ± 127.11	-49.95	$P < 0.05$
Vd/f (l/kg)	0.2023 ± 0.0632	0.15531 ± 0.0578	-23.24	$P < 0.05$	0.2233 ± 0.2893	0.32879 ± 0.2098	47.2414	NS
Cl/f (l/hr/kg)	0.04138 ± 0.0269	0.0249 ± 0.0176	-39.82	$P < 0.05$	0.0053 ± 0.0027	0.0115 ± 0.0057	117.326	$P < 0.05$

9 Adverse effects

Ten adult healthy Thai male volunteers were enrolled and completed in this study. No serious side effects were observed after taking 200 mg of ketoconazole. After a single oral dose of risperidone, all subjects were somnolent, three subjects reported dizziness and one subject had mild headache (Table 25). The symptoms were subsides within one day and did not required any specific treatment. Moreover, all subjects were well tolerated to the drug throughout the study.

Table 25 Adverse effects were observed in ten subjects after receiving a single oral dose of 2 mg risperidone alone, and after pretreatment with 200 mg ketoconazole orally for 3 days

| Adverse Effects	Number of Case (%)	
	Risperidone alone	Risperidone after ketoconazole
Somnolence	10 (100%)	10 (100%)
Dizziness	3 (30%)	5 (50%)
Mild headache	1 (10%)	2 (20%)
Syncope	0 (0%)	0 (0%)

Subject 1
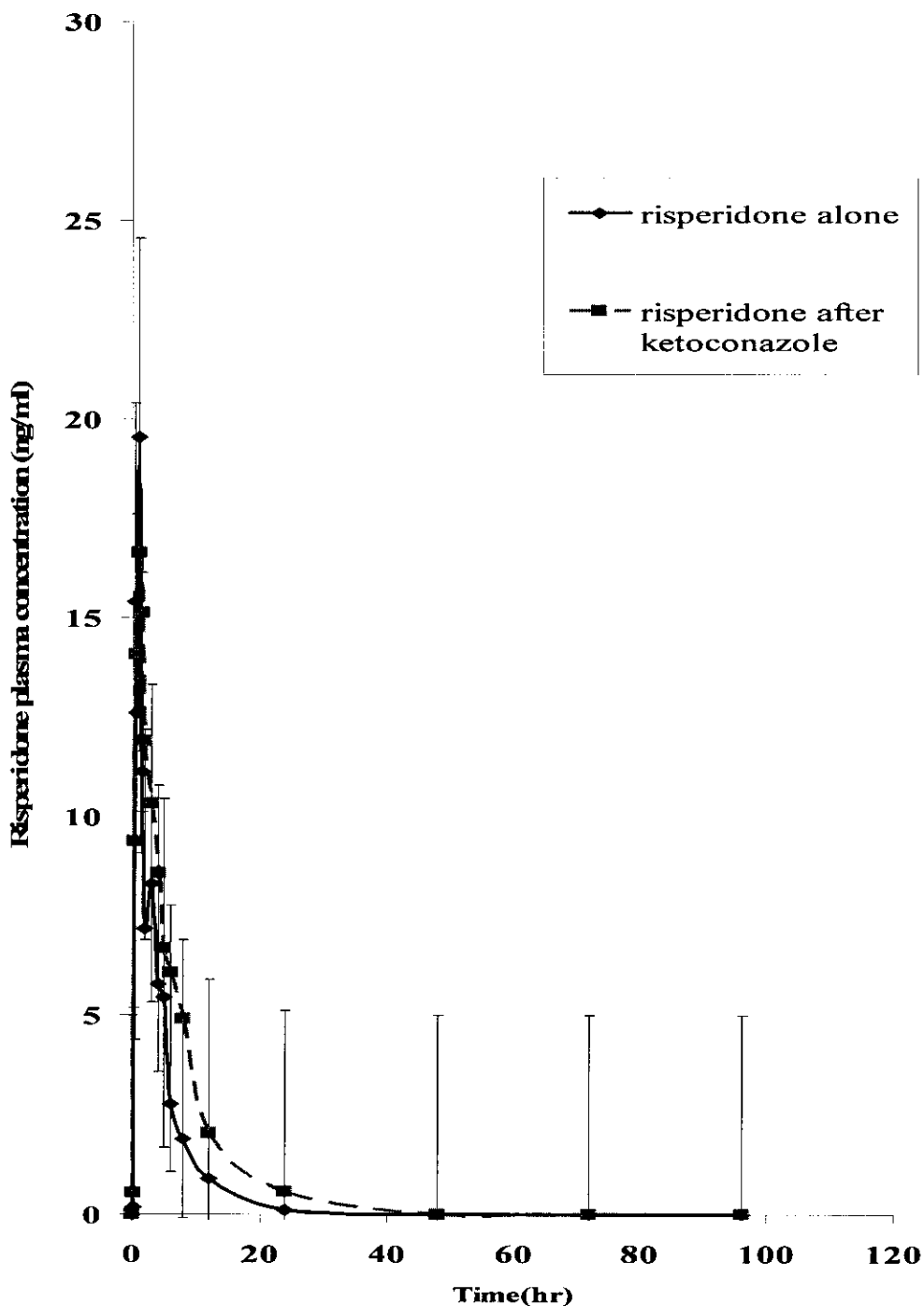
Figure 11 Plasma concentration-time profiles of risperidone after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.1
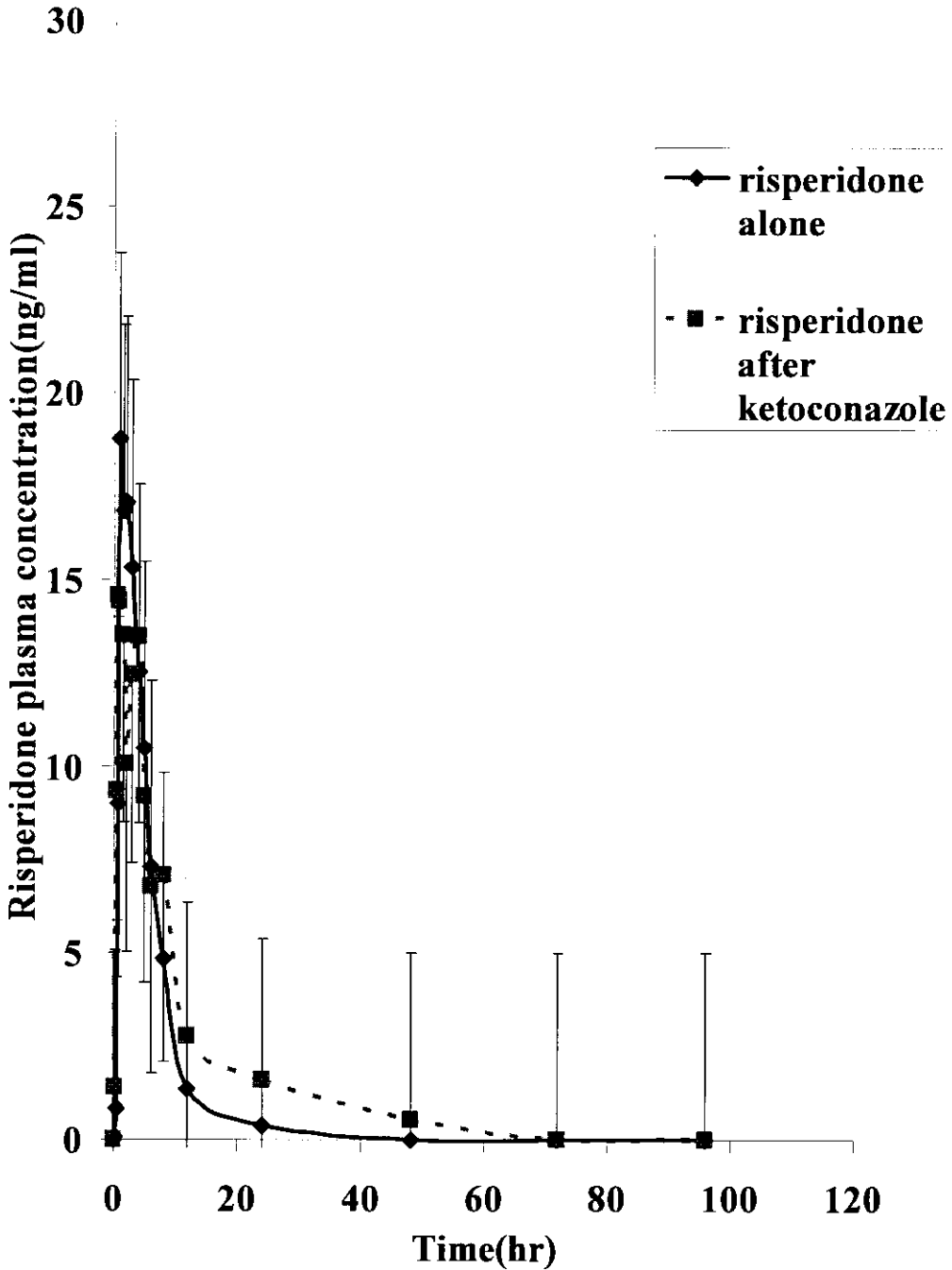
Subject 2
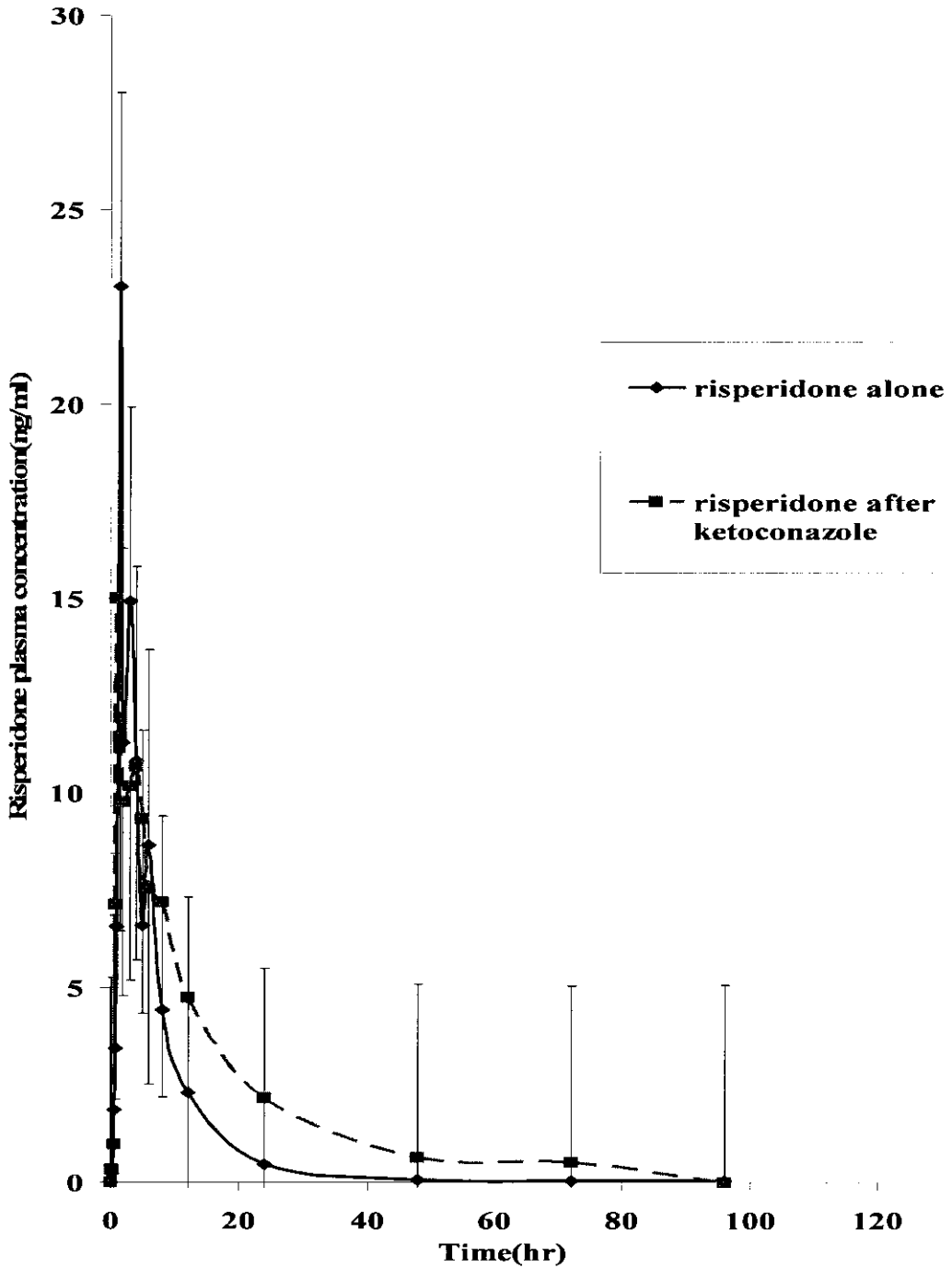
Figure 12 Plasma concentration-time profiles of risperidone after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.2

Subject 3
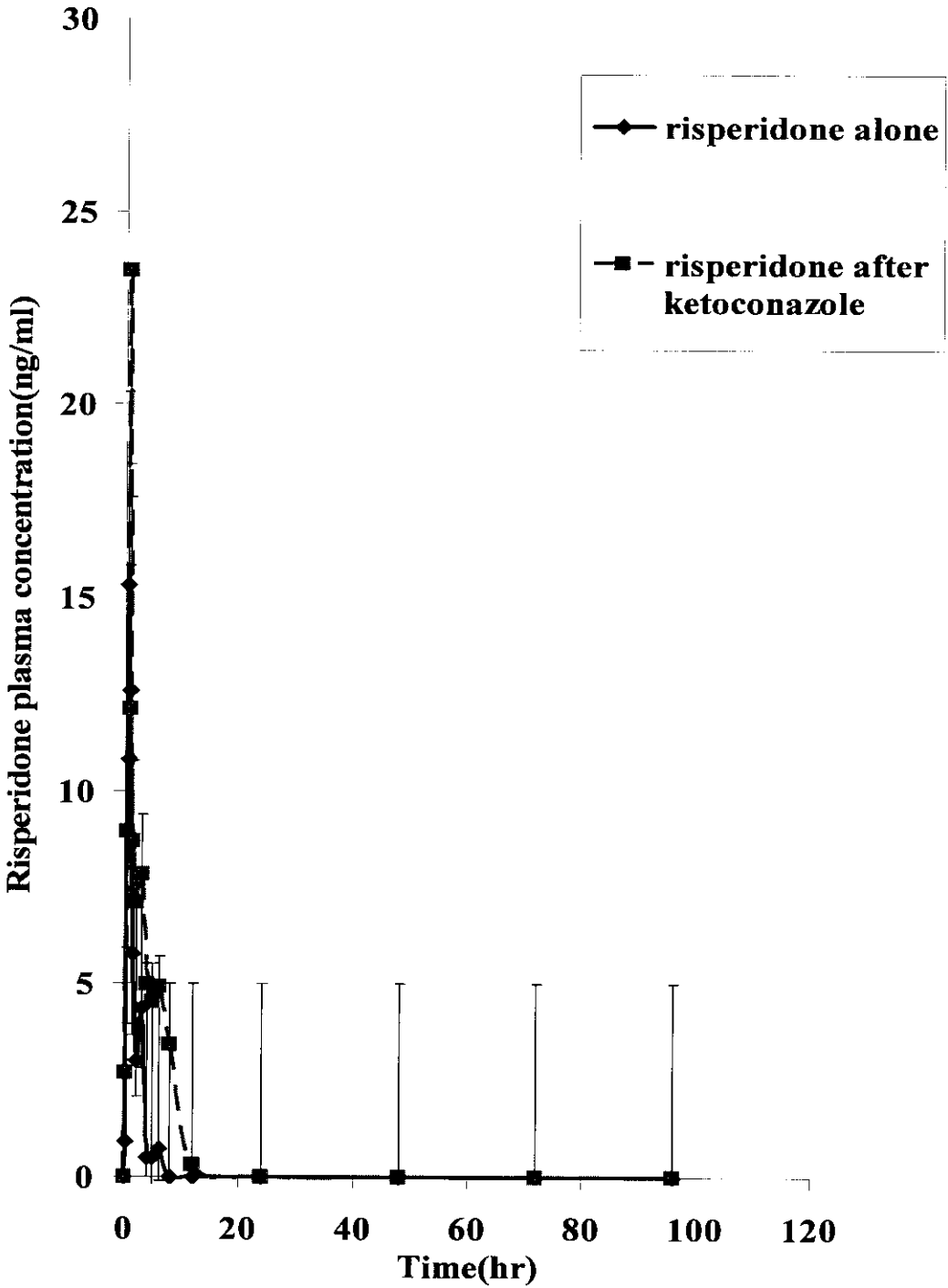
Figure 13 Plasma concentration-time profiles of risperidone after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.3
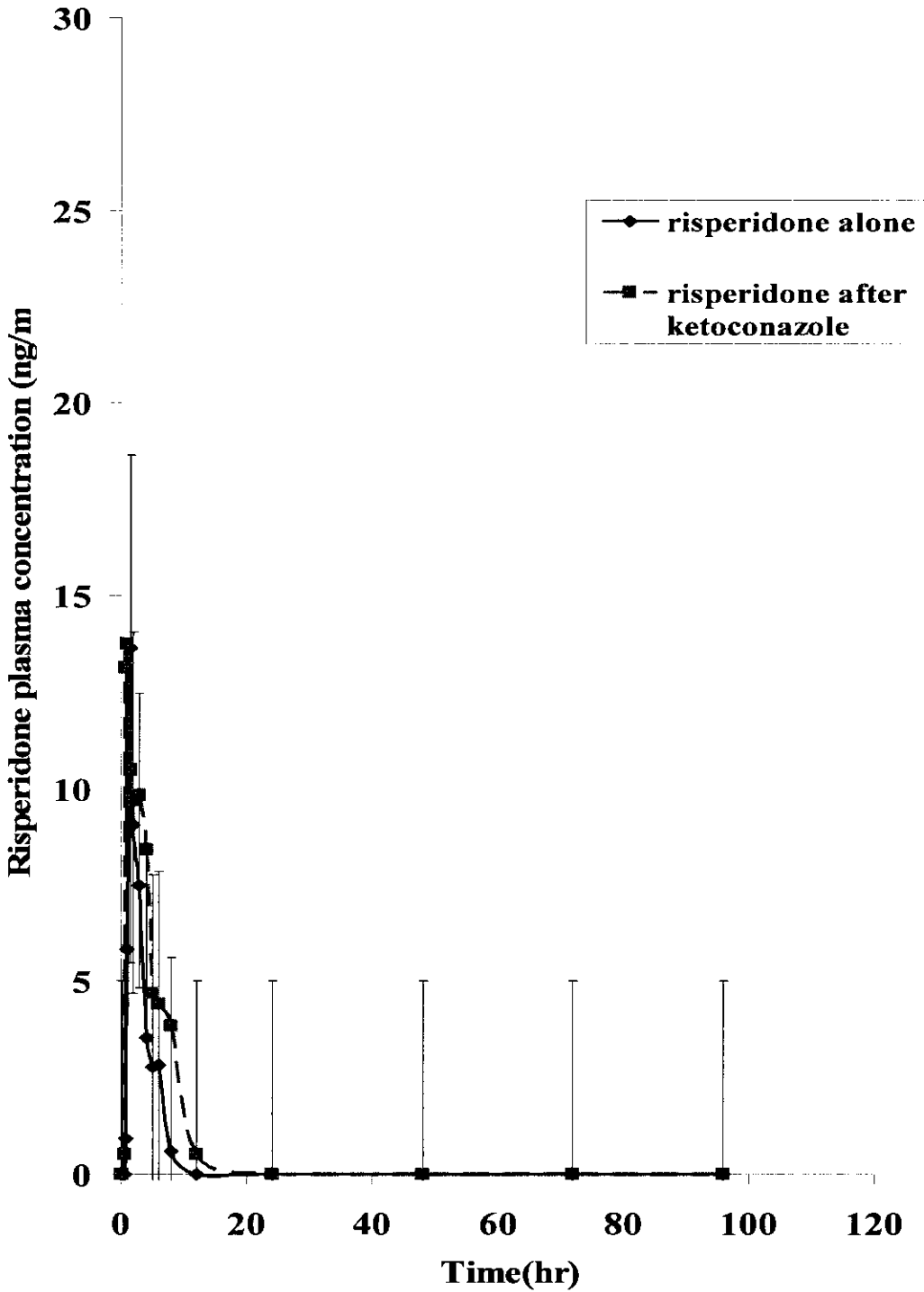
Figure 14 Plasma concentration-time profiles of risperidone after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.4
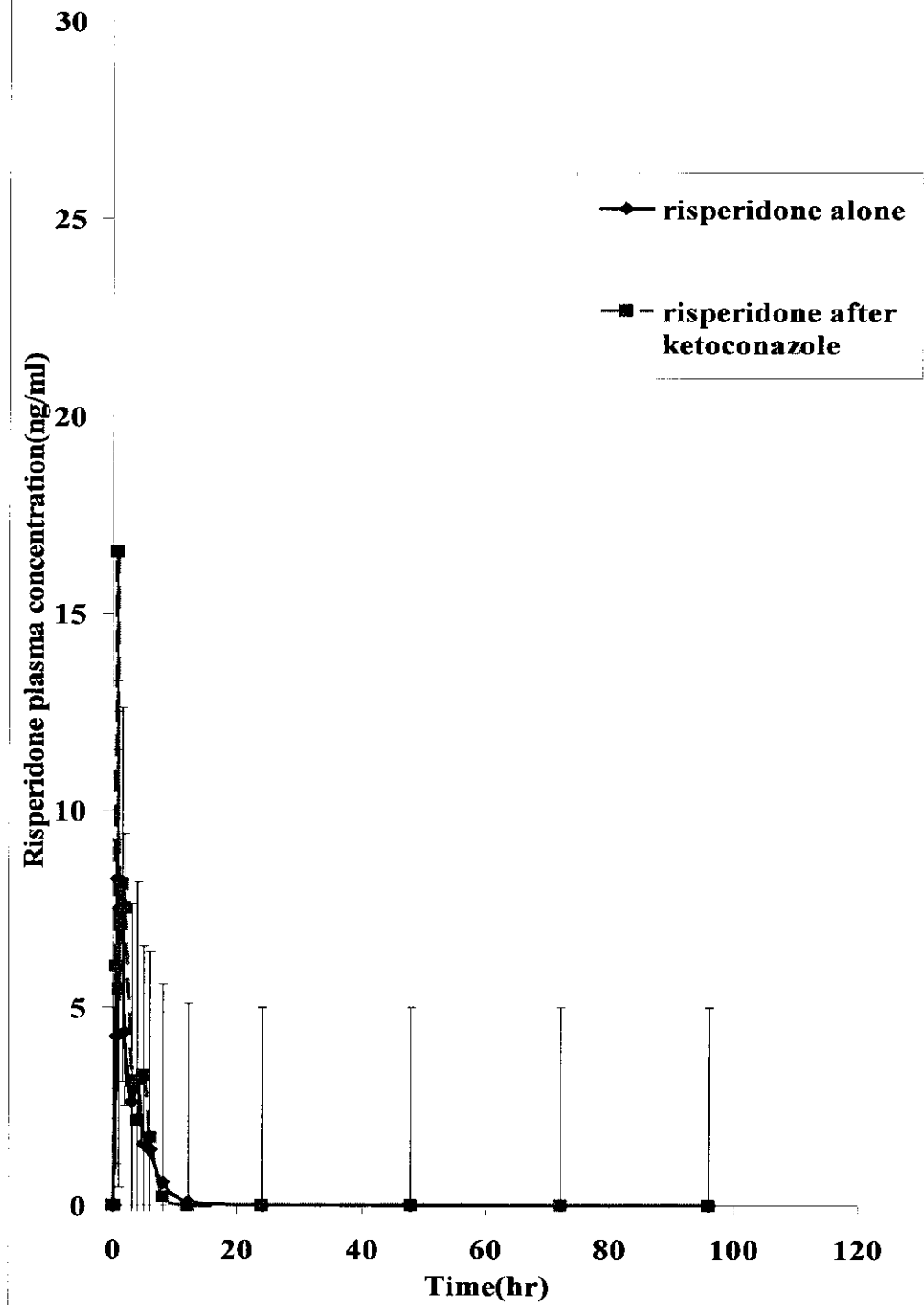
Figure 15 Plasma concentration-time profiles of risperidone after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.5
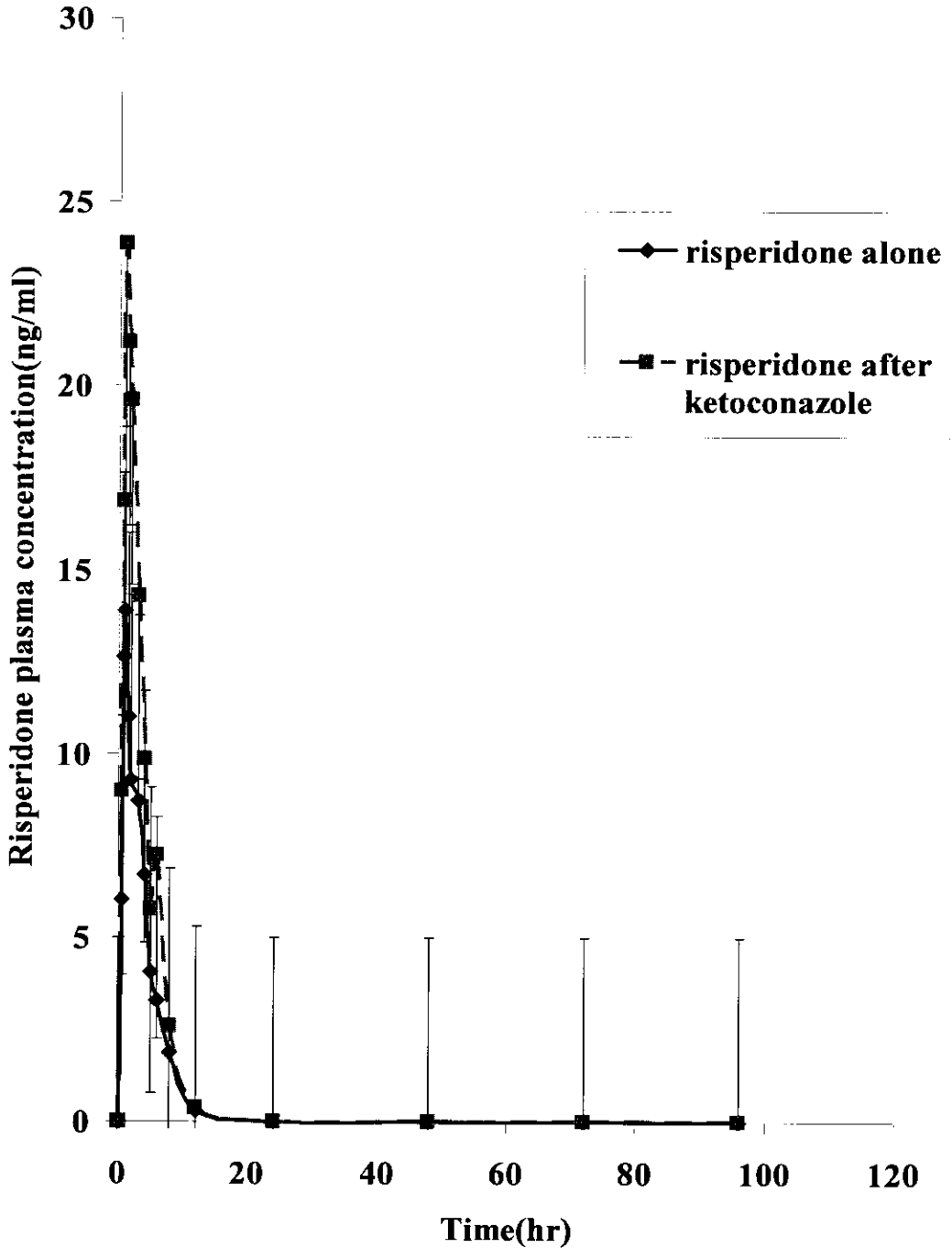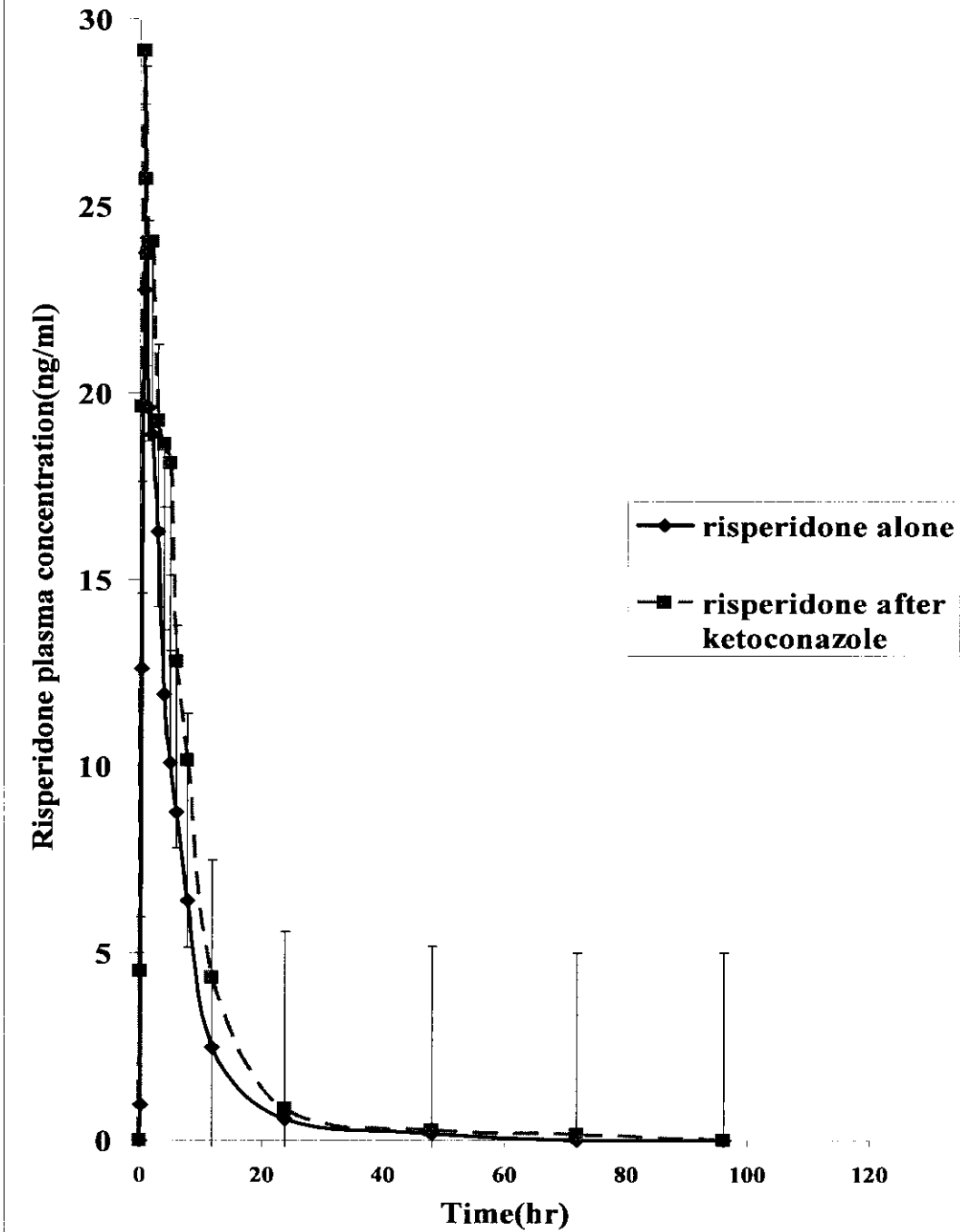
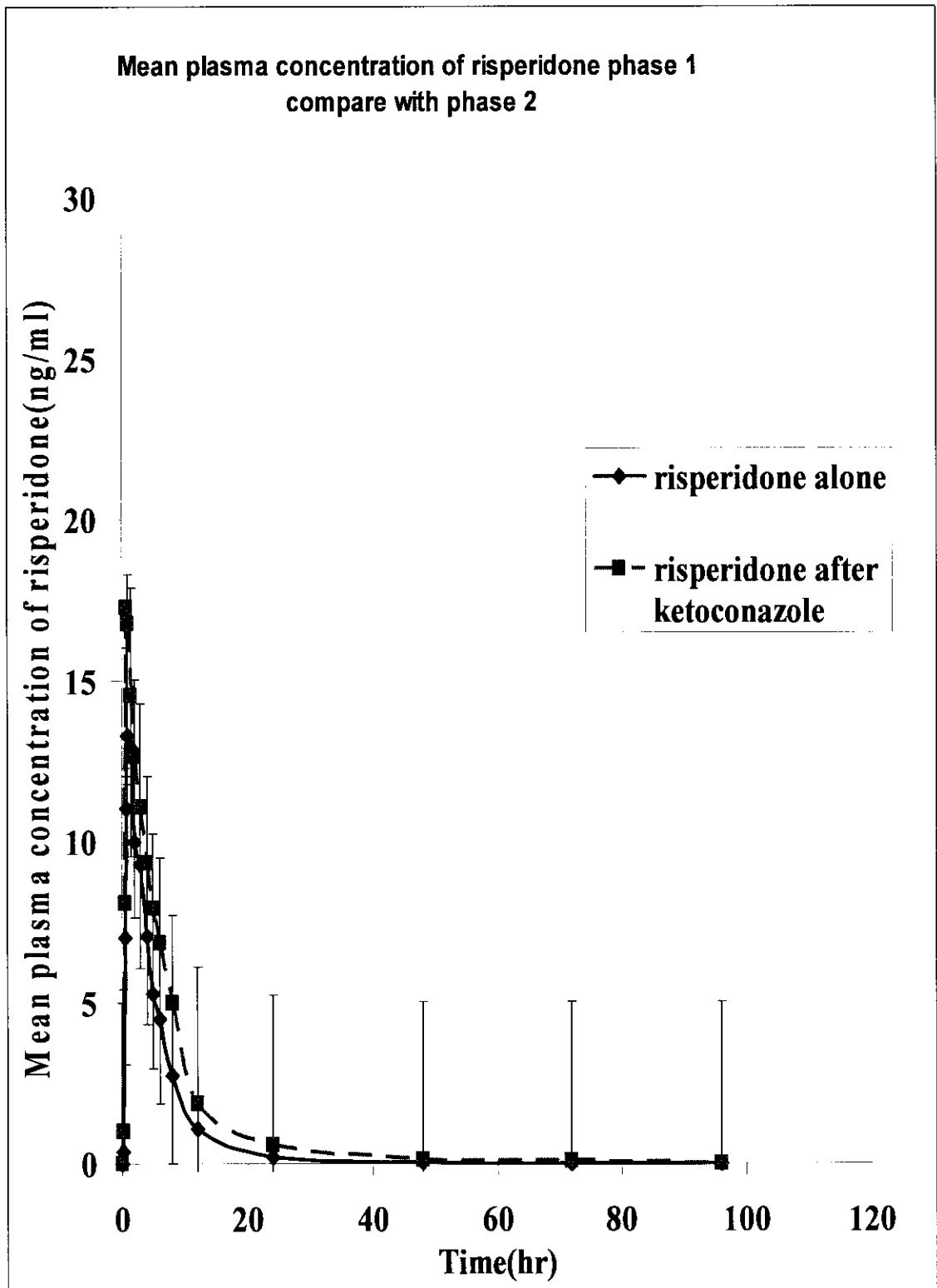# Subject 6

Figure 16 Plasma concentration-time profiles of risperidone after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.6
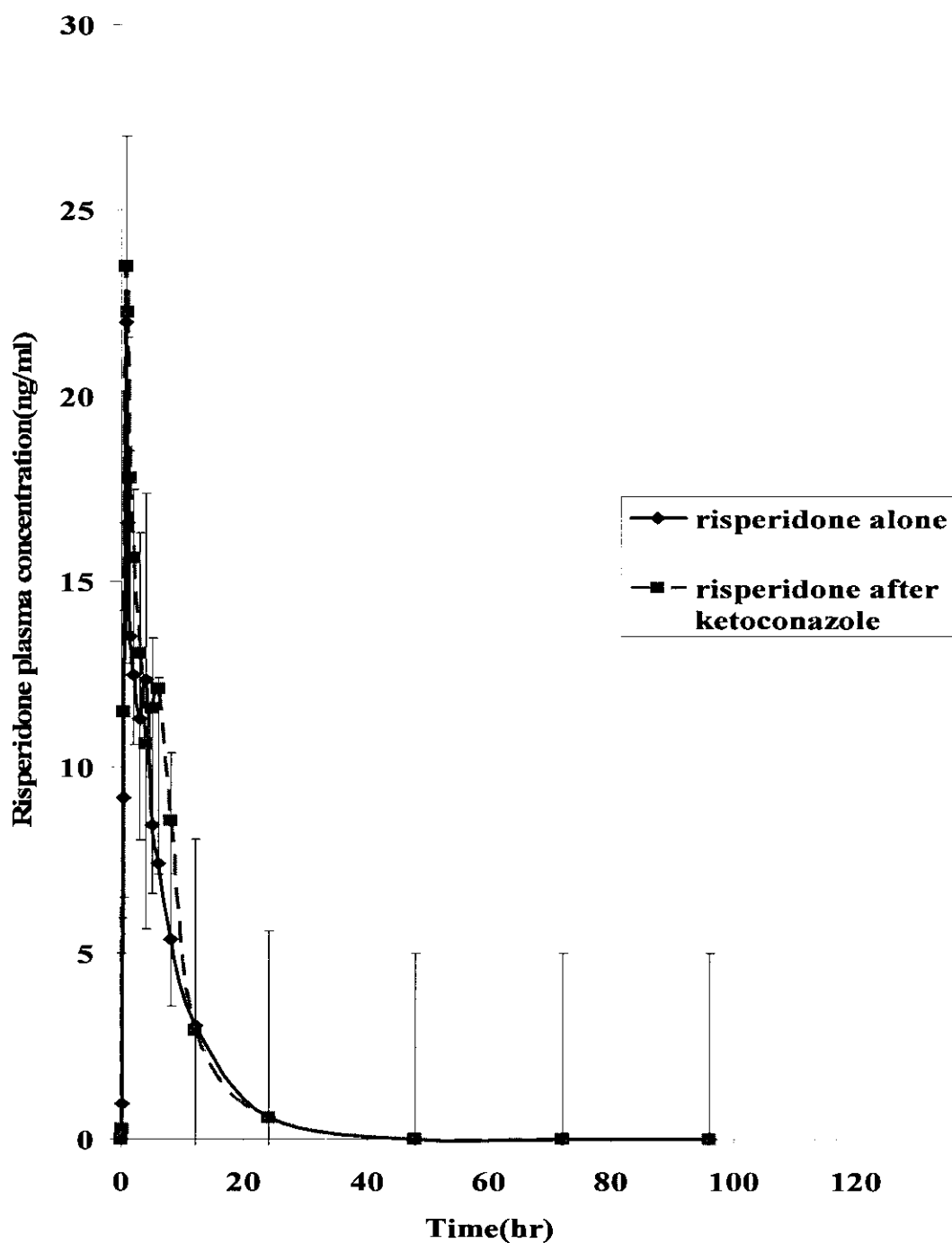
Subject 7

Figure 17 Plasma concentration-time profiles of risperidone after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.7
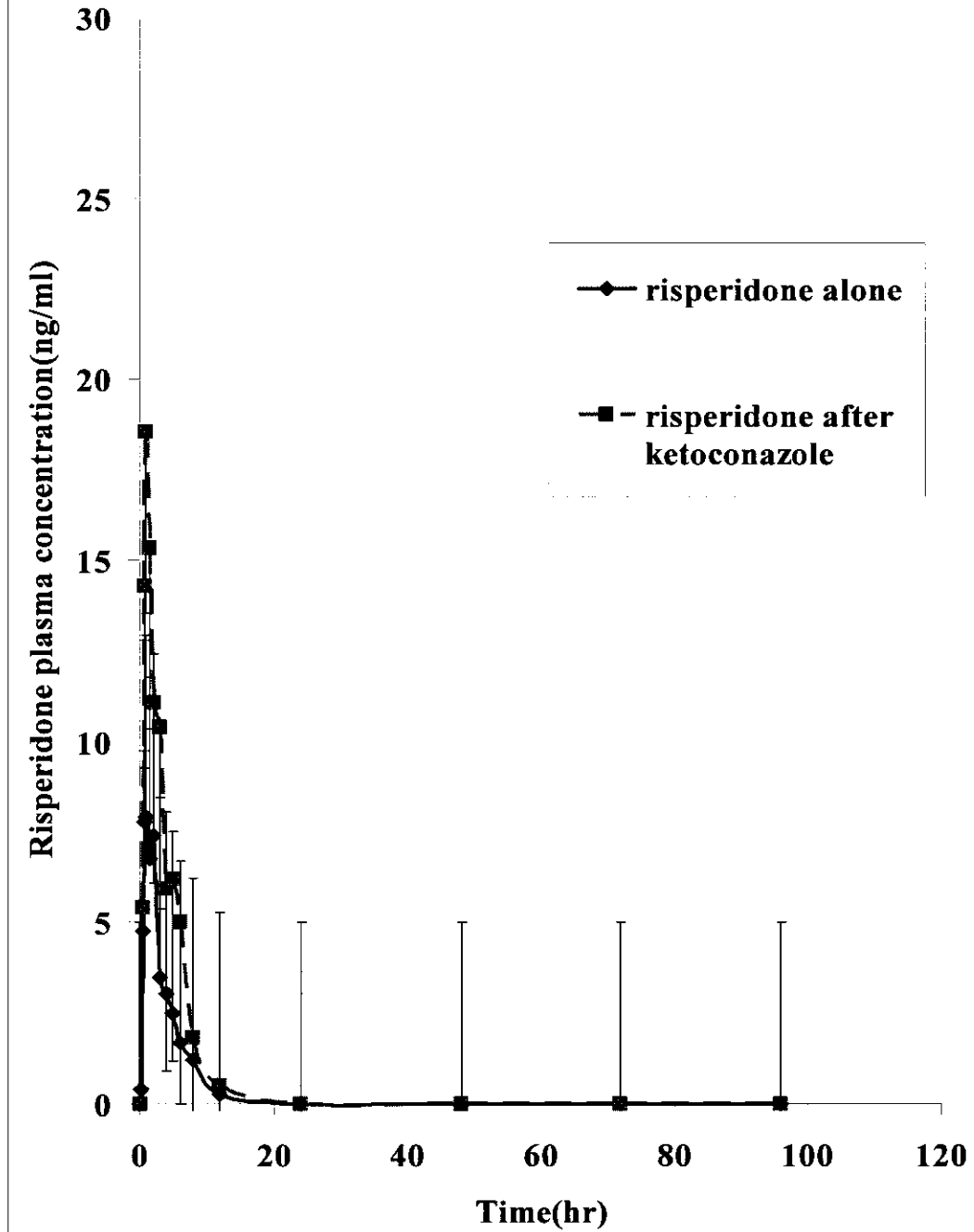
Figure 18 Plasma concentration-time profiles of risperidone after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.8

Subject 9

Figure 19 Plasma concentration-time profiles of risperidone after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.9
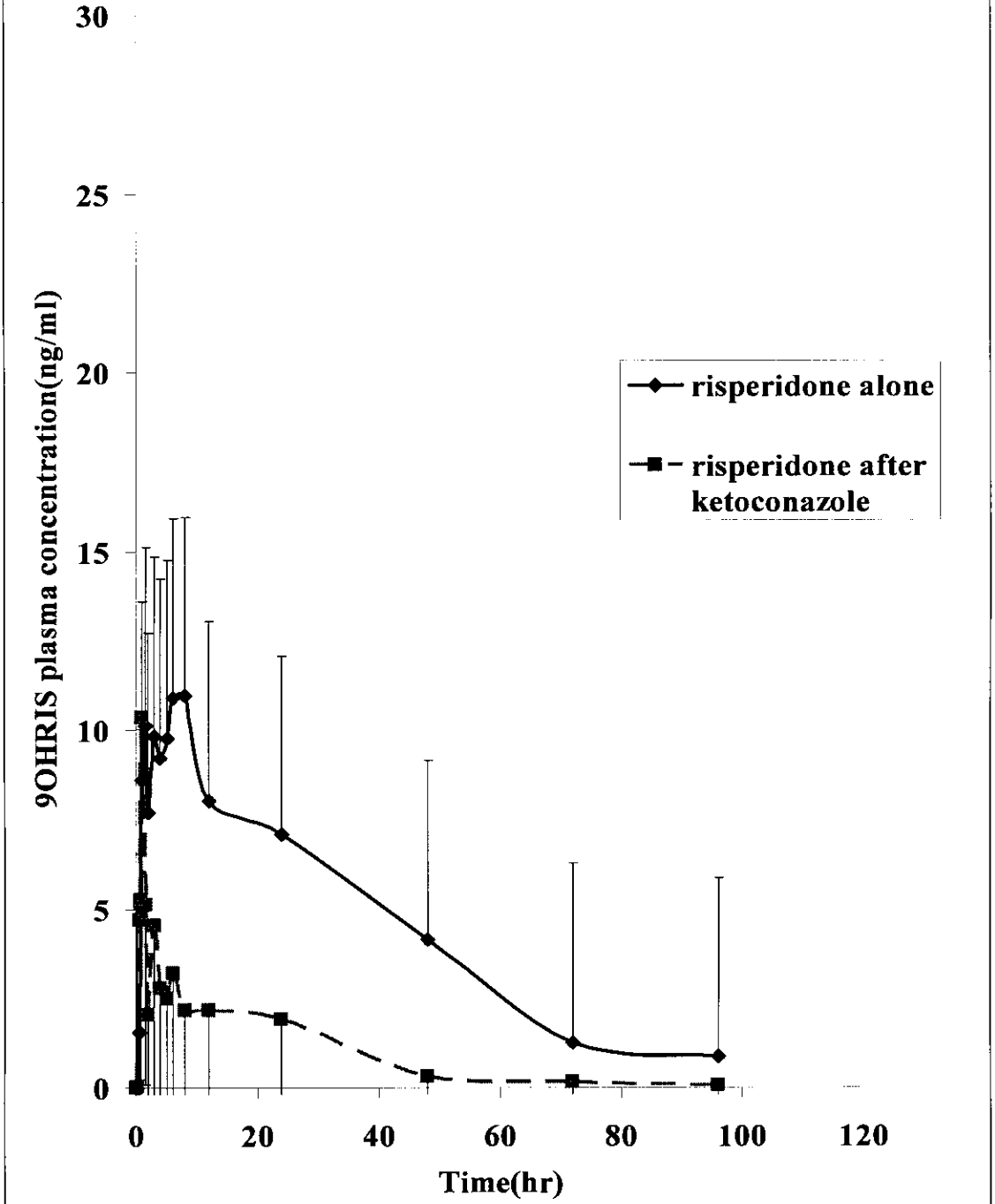
Subject 10

Figure 20 Plasma concentration-time profiles of risperidone after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.10

Figure 21 Mean peak plasma concentration-time profiles of risperidone after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.1-10
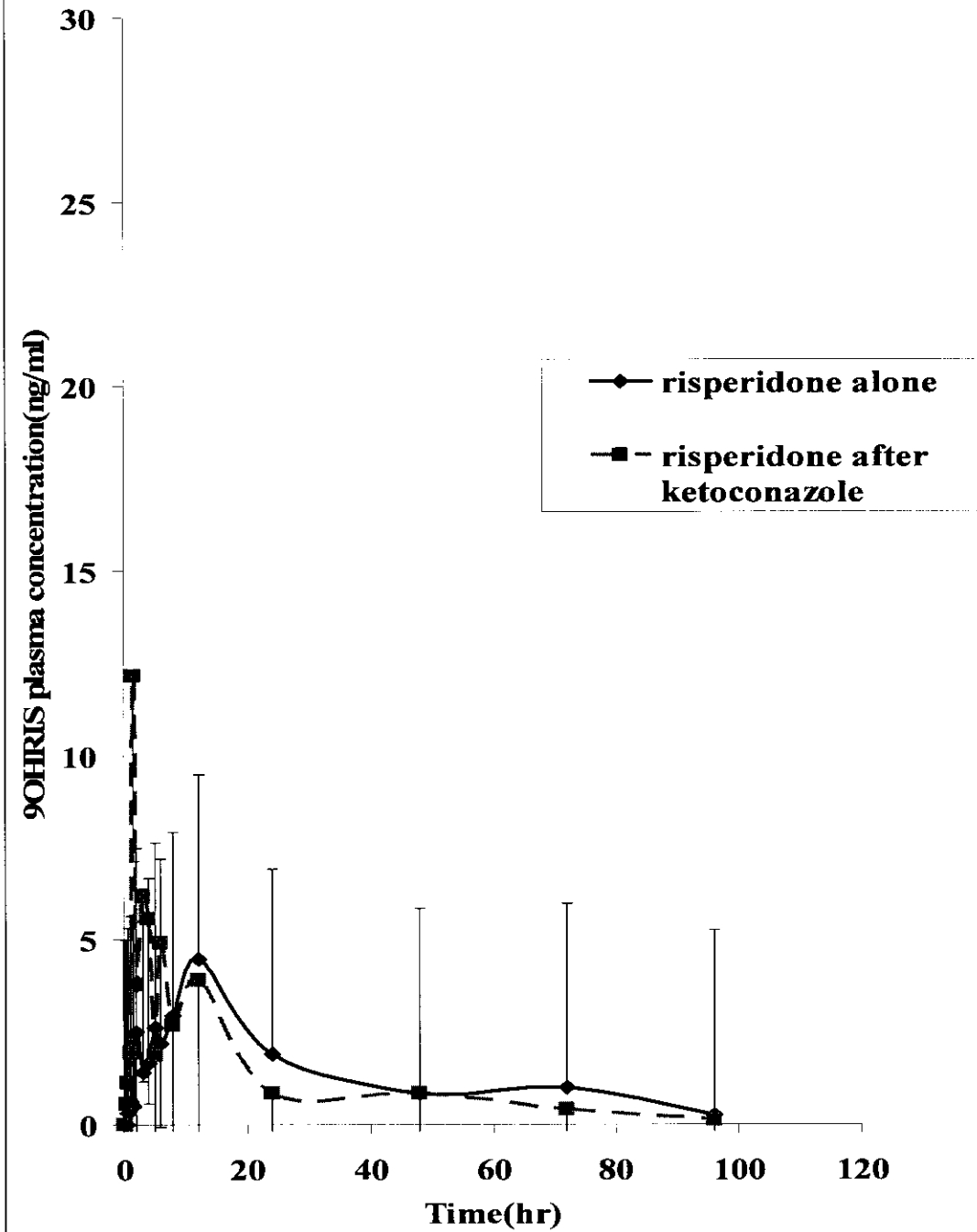
Subject 1

Figure 22 Plasma concentration-time profiles of 9-hydroxyrisperidone (active metabolite of risperidone) after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.1
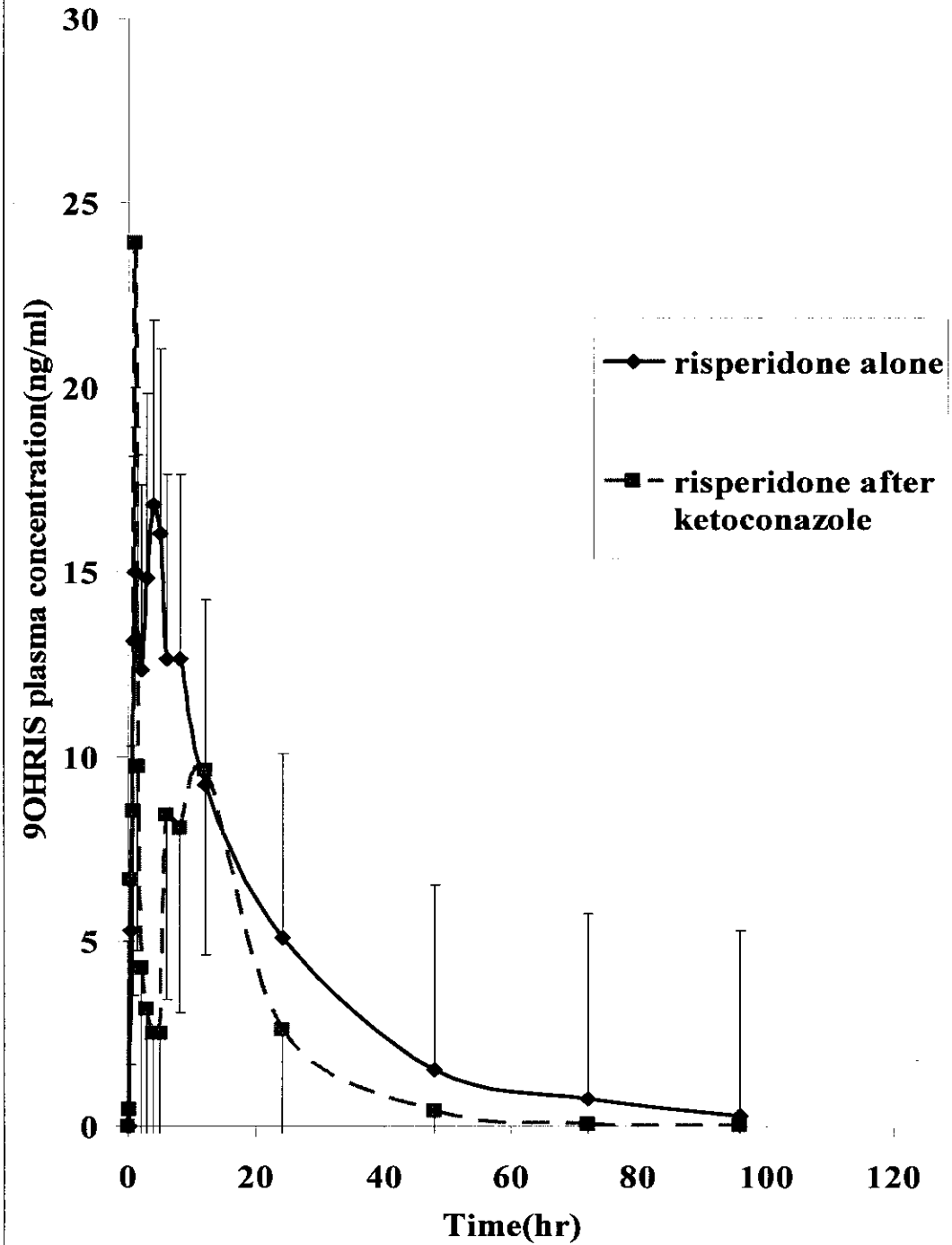
Figure 23 Plasma concentration-time profiles of 9-hydroxyrisperidone (active metabolite of risperidone) after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.2

Figure 24 Plasma concentration-time profiles of 9-hydroxyrisperidone (active metabolite of risperidone) after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.3
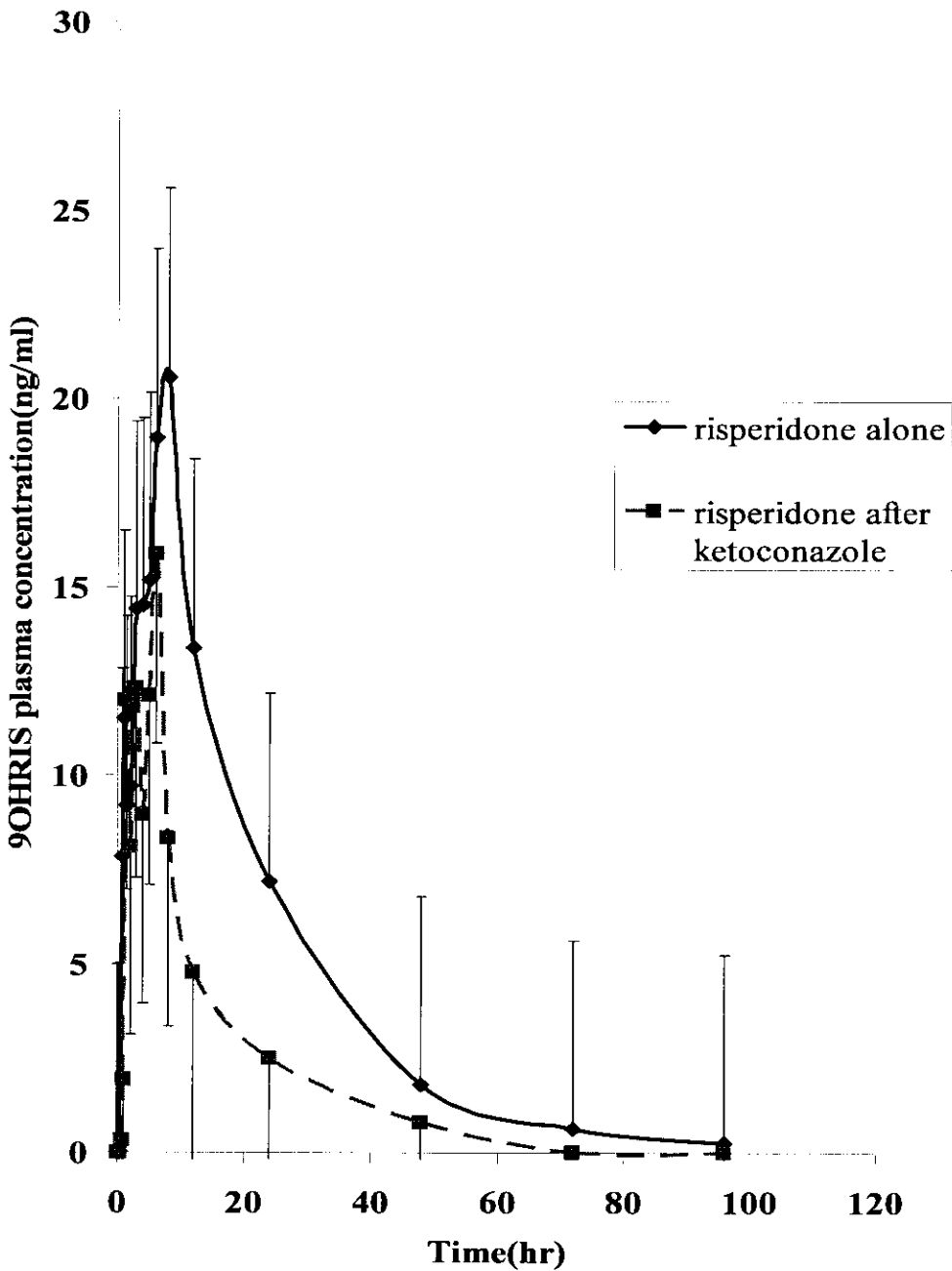
Subject 4

Figure 25 Plasma concentration-time profiles of 9-hydroxyrisperidone (active metabolite of risperidone) after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.4
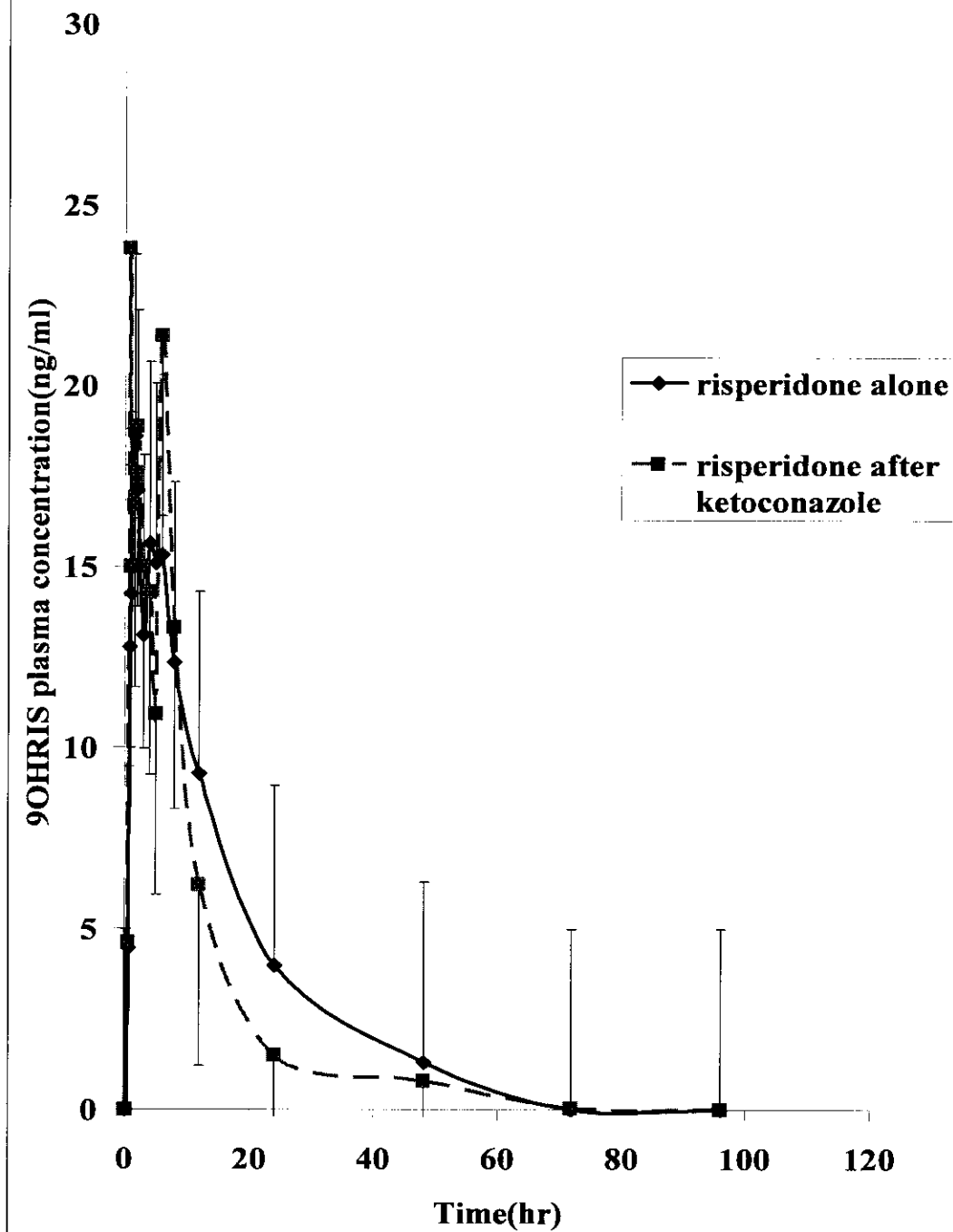
Subject 5

Figure 26 Plasma concentration-time profiles of 9-hydroxyrisperidone (active metabolite of risperidone) after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.5

Subject 6

Figure 27 Plasma concentration-time profiles of 9-hydroxyrisperidone (active metabolite of risperidone) after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.6
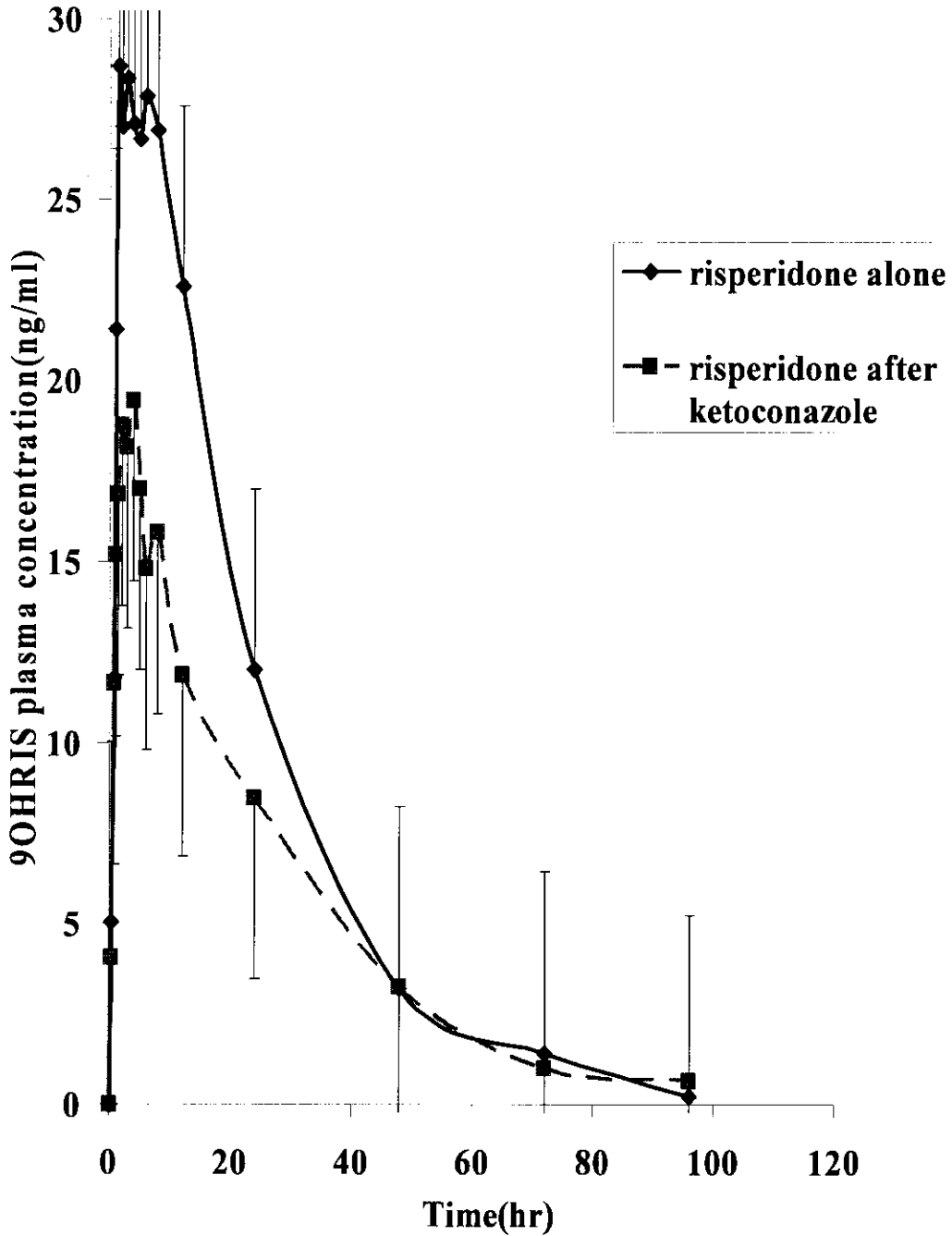
Subject 7

Figure 28 Plasma concentration-time profiles of 9-hydroxyrisperidone (active metabolite of risperidone) after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.7

Figure 29 Plasma concentration-time profiles of 9-hydroxyrisperidone (active metabolite of risperidone) after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.8
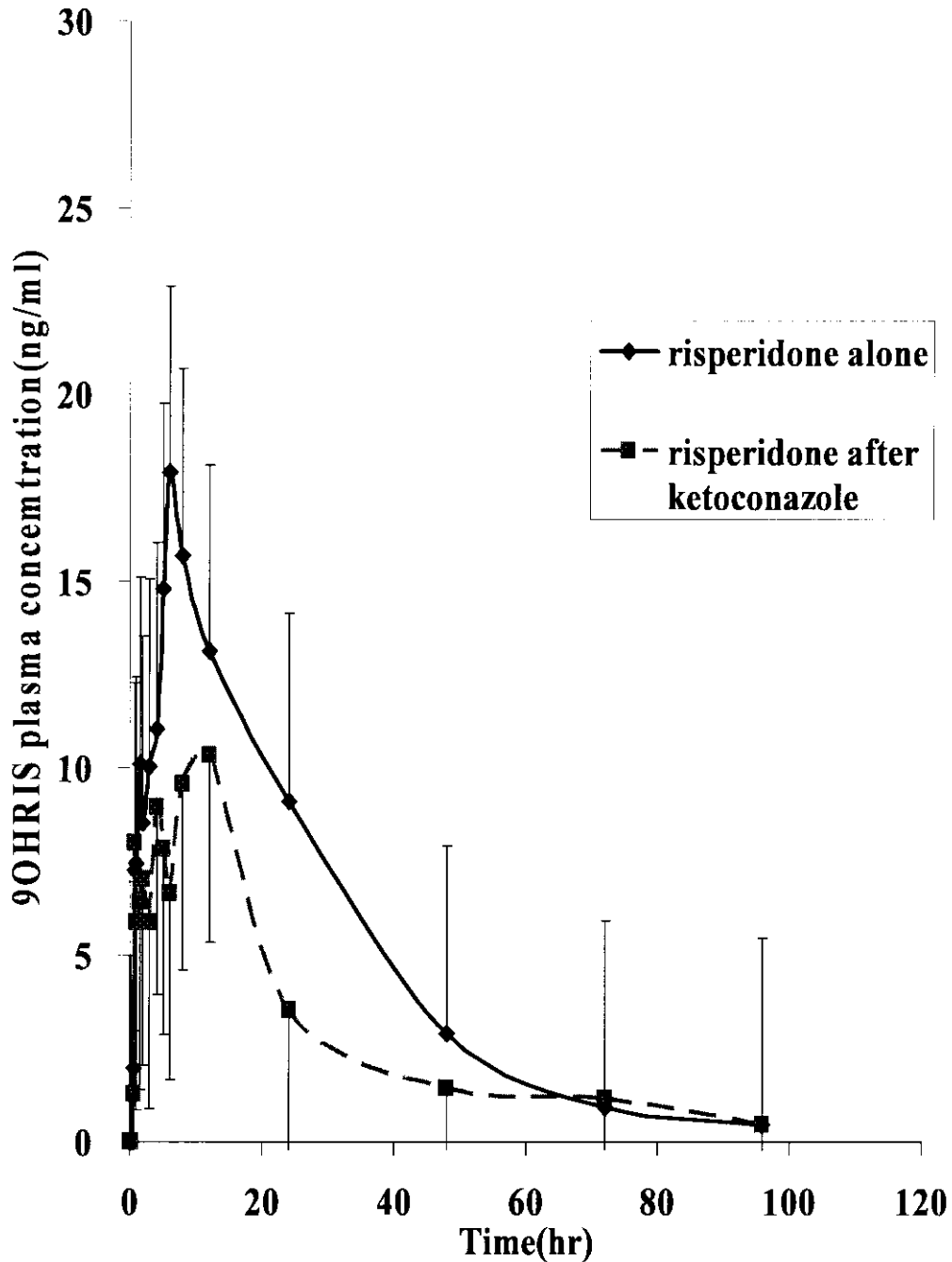
Subject 9

Figure 30 Plasma concentration-time profiles of 9-hydroxyrisperidone (active metabolite of risperidone) after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.9
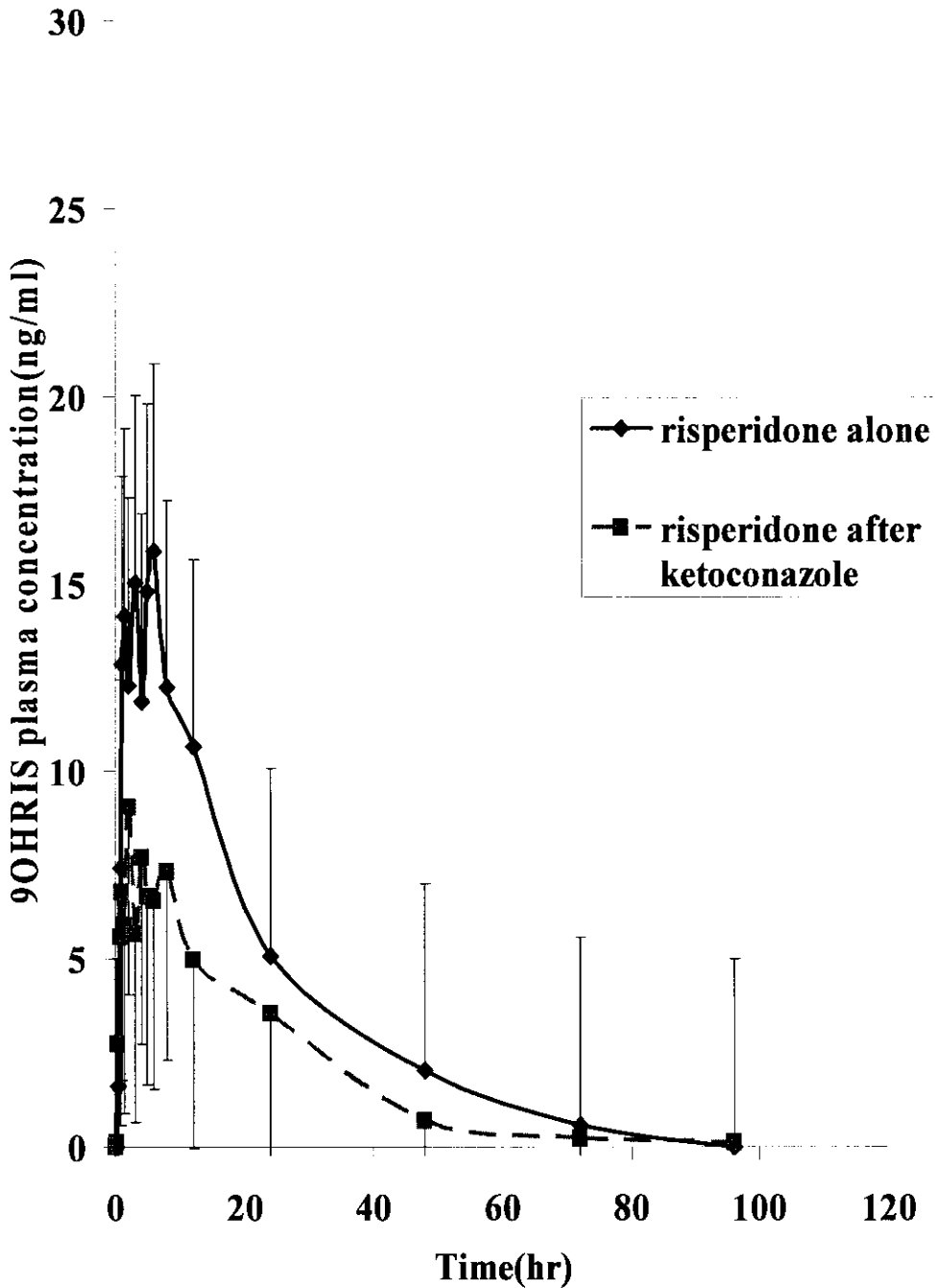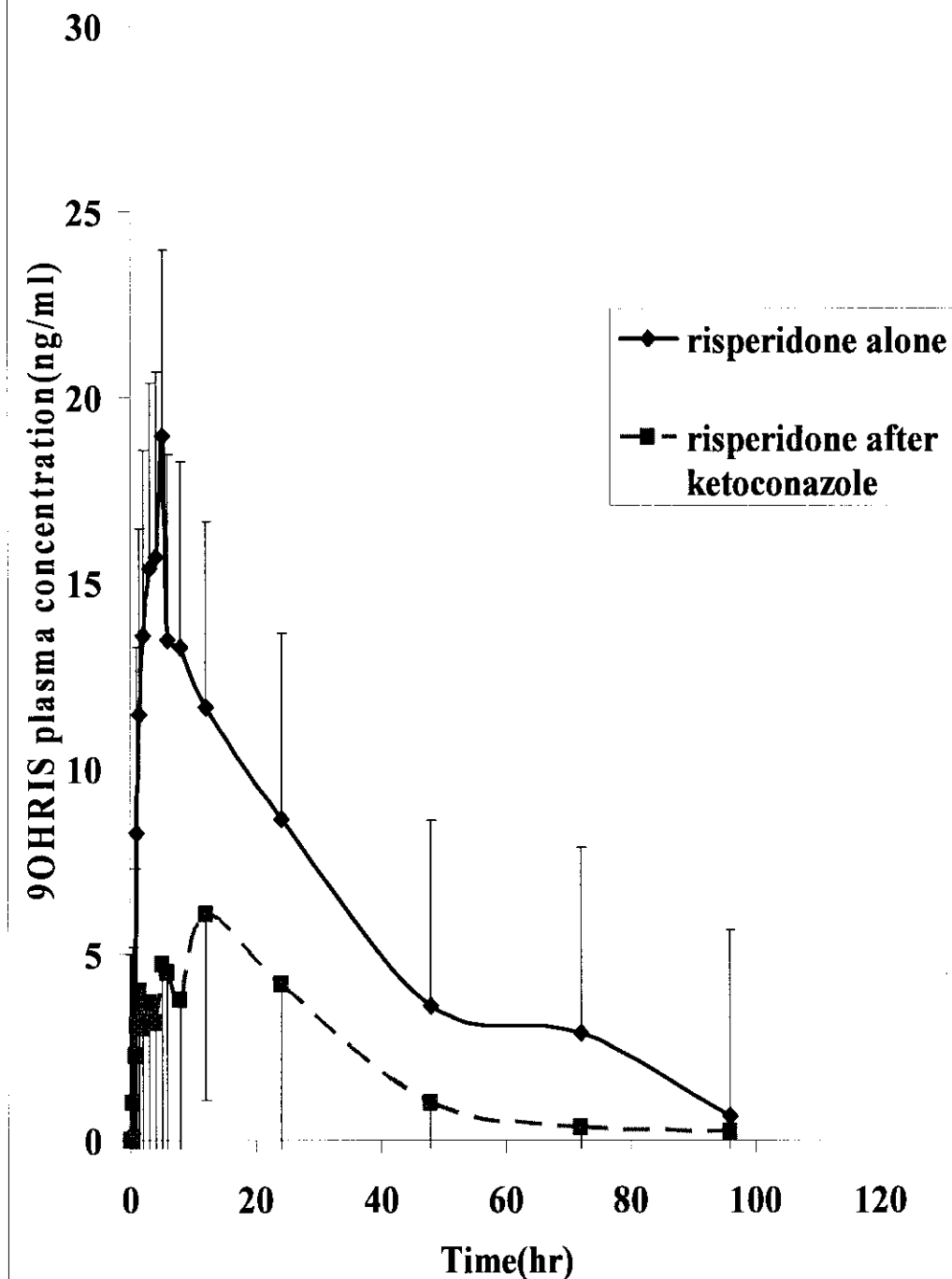
Subject 10

Figure 31 Plasma concentration-time profiles of 9-hydroxyrisperidone (active metabolite of risperidone) after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.10

Mean plasma concentration of 9OHRIS phase 1 compare with phase 2.

Figure 32 Mean peak plasma concentration-time profiles of 9-hydroxyrisperidone after a single oral administration of 2 mg risperidone alone and risperidone after ketoconazole to subject No.1-10

DISCUSSION AND CONCLUSION
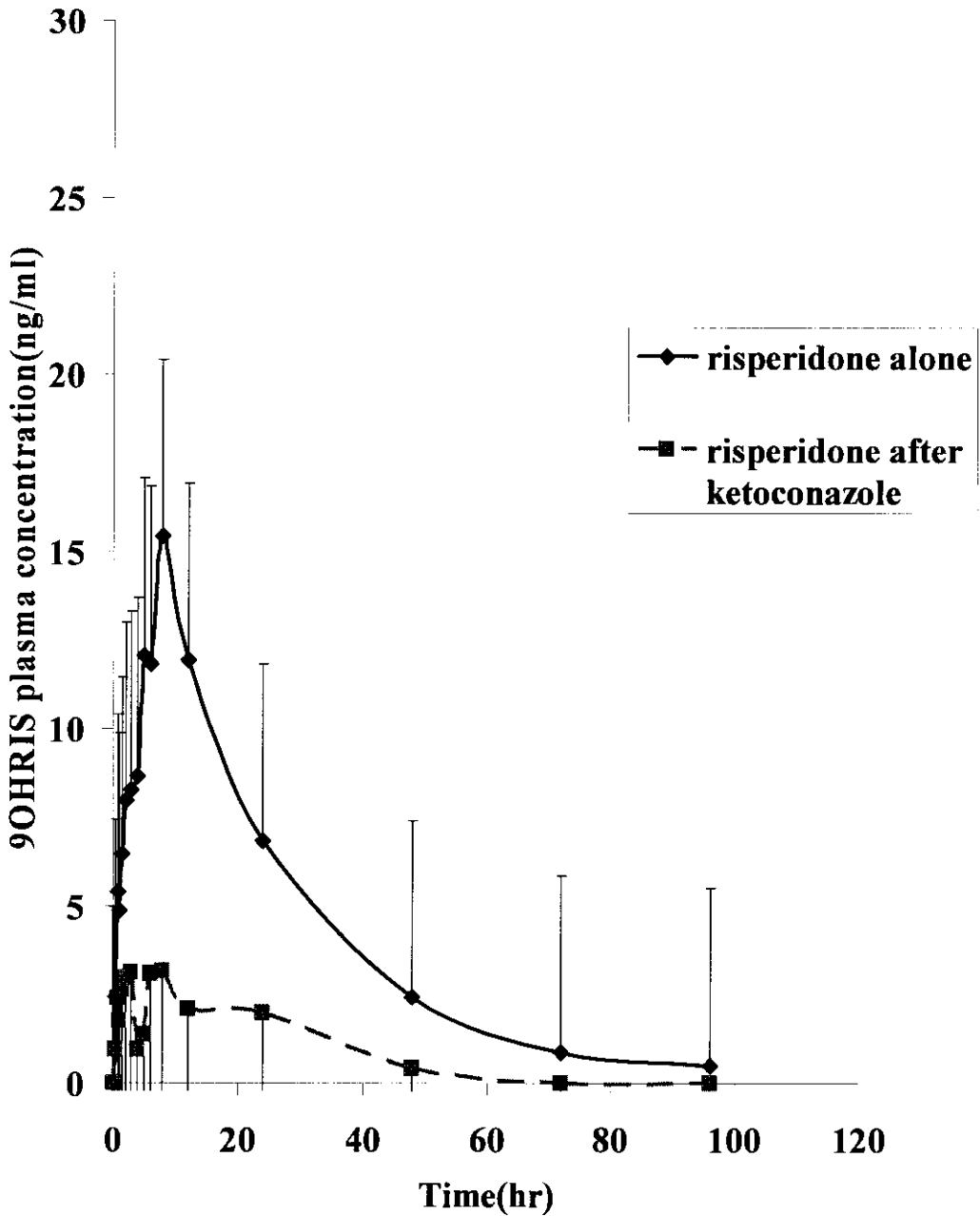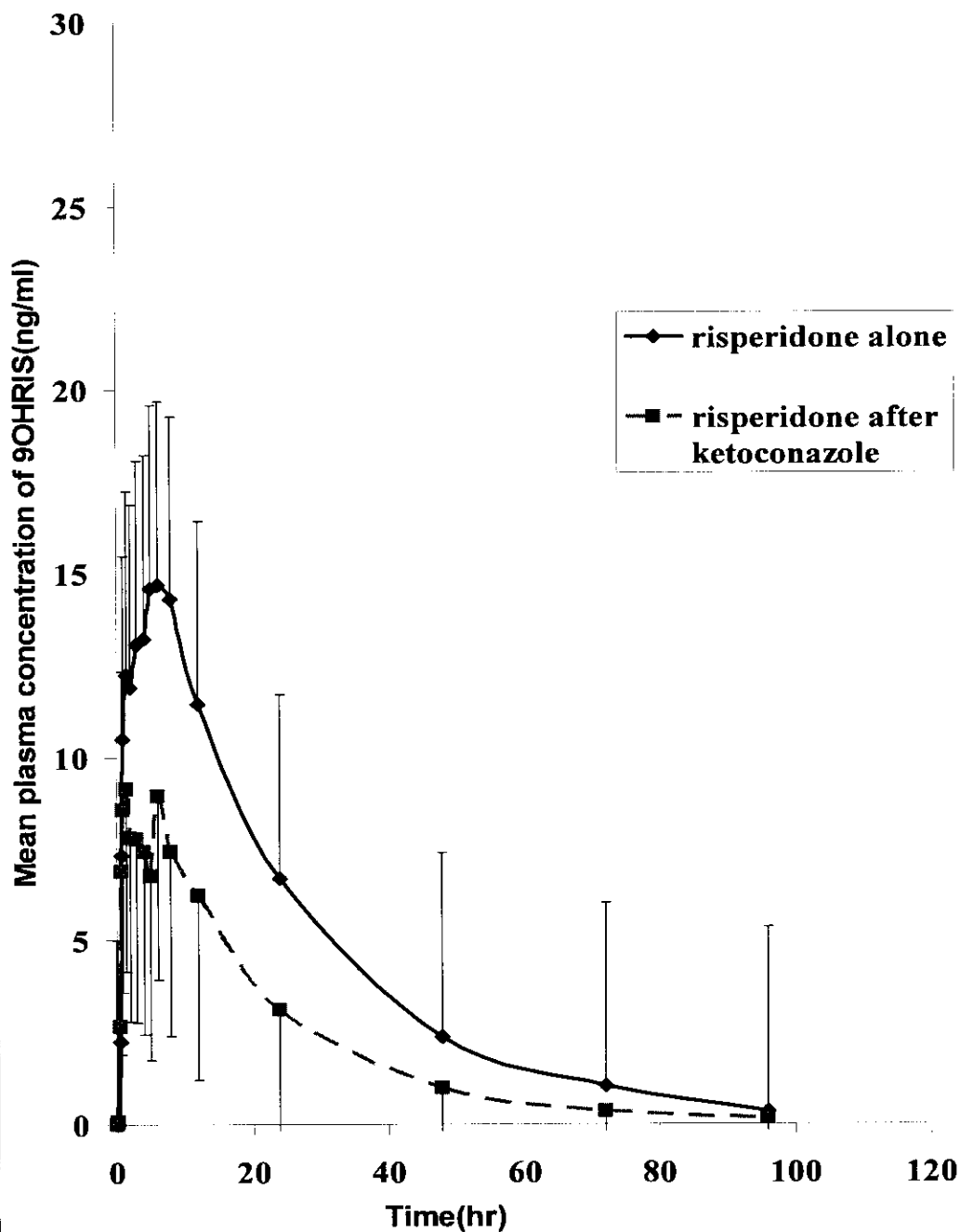
Validation of the analytical method was performed in accordance to Remmerie *et al*, (2003). The specificity test was done to ensure the authenticity of the results for the sample analysis that no endogenous compounds or other impurities interfered with the assay. RIS, 9OHRIS and CBZ were separated with the C18-chromatographic column and provided the retention times of 4.3, 3.3 and 7.2 min, respectively. All interested peaks were completely separated using isocratic elution mobile phase. No interferences of endogenous substances were observed (Figure 7 to Figure 9). The calibration curves of 9OHRIS and RIS were shown in Figure 10. The linear response over the range of concentration used in the assay procedure with the coefficient of determination (r^2) greater than 0.99 was observed. The equations for mean of the five calibration curves for each analyte of RIS and 9OHRIS were $y = 0.3474x + 0.0654$ ($r^2 = 0.9981$) and $y = 0.1631x + 0.0317$ ($r^2 = 0.9983$), respectively (Table 4 and 5). The LLOQ of RIS was 0.1 ng/ml and 9OHRIS was 0.3 ng/ml as shown in Table 6 and 7. Accuracy and precision results were summarized in Table 8-11. The accuracy of the within-run study of risperidone and 9-hydroxyrisperidone were 93.29 % to 103.65% and 92.26% to 100.95% respectively and the precision (%CV) were 3.36% to 6.28% and 3.04% to 8.20%, respectively (Table 8-9). The accuracy of the between-run of risperidone and 9-hydroxyrisperidone were 99.57% to 102.17 and 96.79% to 100.06%, respectively and the precision (%CV) were 3.87% to 5.78% and 1.53% to 7.14%, respectively (Table 10-11). For the precision, the %RSD of within-run and between–run of both 9OHRIS and RIS were less than 15%.

The present results revealed that when a single oral dose of risperidone was administered either without or with ketoconazole for 3 days, plasma concentrations of risperidone could be detected in all subjects. In the ketoconazole pretreated group, the mean C_{max} of risperidone was not significantly increased by 17.42% (19.50 ± 5.15 from 16.60 ± 5.74 ng/ml; $p > 0.05$), (1.17 fold). The T_{max} of risperidone was also not significantly decreased by 12.5% (0.875 ± 0.13 vs 1 ± 0.31 hr; $p > 0.05$), (1.14 fold). The $T_{1/2}$ of risperidone was significantly increased by 32.8% (6.46 ± 5.32 vs 4.87 ± 3.23 hr ; $p < 0.05$), (1.33 fold) and the AUC_{0-48} and AUC_{0-96} of risperidone were significantly increased by 53.7% (109.47 ± 60.85 vs 71.21 ± 45.56 ng/l/hr ; $p < 0.05$), (1.54 fold) and 55.37% (113.71 ± 63.31 vs 73.19 ± 46.35 ng/l/hr ; $p < 0.05$), (1.55 fold), respectively. The Vd/f and Cl/f of risperidone were significantly decreased by 23.24% (0.15531 ± 0.0578 vs 0.2023 ± 0.0632 l/kg; $p < 0.05$), (1.3 fold) and 39.82% (0.0249 ± 0.01766 vs 0.04138 ± 0.02699 l/hr/kg ; $p < 0.05$), (1.66 fold), respectively (Table 24).

After pretreatment with ketoconazole prior to risperidone, the C_{max}, T_{max} and $T_{1/2}$ of 9-hydroxyrisperidone were not significantly decreased by 20.32% (13.42 ± 7.13 vs 16.83 ± 6.27 ng/ml; $p > 0.05$), (1.25 fold), 19.58% (4.825 ± 4.47 vs 6 ± 3.22 hr ; $p > 0.05$), (1.24 fold) and 22.48% (19.27 ± 5.77 vs 24.86 ± 13.8 hr ; $p > 0.05$), (1.29 fold), respectively but the Cl/f of 9-hydroxyrisperidone was significantly increased by 117.32% (0.0115 ± 0.0057 vs 0.0053 ± 0.0027 l/hr/kg ; $p < 0.05$), (2.17 fold). These changes led to corresponding decrease in the AUC_{0-48} and AUC_{0-96} of 9-hydroxyrisperidone. They were significantly decreased by 50.05% (211.65 ± 123.67

vs 423.74 ± 163.46 ng/l/hr; $p < 0.05$), (2 fold) and 49.95% (220.76 ± 127.11 vs 441.08 ± 171.63 ng/l/hr; $p < 0.05$), (2 fold), respectively. Anyway, the Vd/f was not significantly increased by 47.24% (0.32879 ± 0.2098 vs 0.2233 ± 0.2893 l/kg; $p > 0.05$), (1.47 fold) (Table 24).

The C_{max} and T_{max} of risperidone and 9-hydroxyrisperidone did not change when compared with control group in phase 1. But this study showed that the AUC_{0-48} and AUC_{0-96} of risperidone were significantly increased. Because ketoconazole can inhibit CYP3A4, thus risperidone could not completely be metabolized to 9-hydroxyrisperidone. The change led to a significantly decreased in AUC_{0-48} and AUC_{0-96} of 9-hydroxyrisperidone. (Table 24). Pretreatment with ketoconazole for 3 day as in this study can inhibit CYP3A4 which is similar to the previous study (de Bruijn et al., 2001).

The involvement of P-glycoprotein (P-gp) in risperidone disposition has recently been documented (Boulton et al., 2002, Wang et al., 2004 and Nakagami et al., 2005). They showed that in the intestine, P-gp is expressed on the brush-border membrane of enterocytes and pumps the compounds out of the cells from the cytosol to the intestinal lumen. P-gp has been labelled as a transport barrier to the oral absorption of drugs (Leu and Huang, 1995, Fricker et al., 1996, Lown et al., 1997, Kim et al., 1998 and Salphati and Benet, 1998). Moreover, P-gp could act along with CYP3A4 to increase drug presystemic metabolism (Gan et al., 1996, Watkins, 1997, Wacher et al., 1998 and Ito et al., 1999). Two mechanisms have been proposed to explain how P-gp may enhance the extent of intestinal metabolism. According to a first mechanism, P-gp activity induces repeated cycles of secretion into the intestinal lumen, resulting in repeated cycles of absorption. The residence time of a drug in the intestine is increased and its exposure to intestinal CYP3A4 is lengthened prior to systemic absorption (Gan et al., 1996). A second mechanism has been proposed suggesting that P-gp may facilitate the removal of intracellular primary metabolites, thus minimising the potential to produce CYP3A4 inhibition (Lown et al., 1997). The previous study (Etienne et al., 2007) reported the possible involvement of P-gp and CYP 3A4 in risperidone transport was investigated using in vitro and in vivo models. Firstly, uptake studies were performed on a Caco-2/TC7 cell monolayer; the effects of 1 µg/ml risperidone on apparent permeability were determined for secretory and absorptive directions, in the presence or absence of various P-gp and CYP3A4 inhibitors (verapamil, ketoconazole, erythromycin). Secondly, on a conscious rat model, risperidone pharmacokinetic parameters, notably absorption parameters, were determined using compartmental and deconvolution methods. Three groups of seven rats received respectively an IV risperidone dose, an oral risperidone dose (PO group) and the same oral risperidone dose after verapamil administration (POV group). No formation of 9-hydroxyrisperidone was observed on Caco-2 cells after risperidone administration; there was no evidence that intestinal CYP3A4 is involved in risperidone metabolising. Risperidone secretory permeation was higher than absorptive permeation. Verapamil increased risperidone absorption permeation and decreased its secretory permeation. In rats, verapamil led to an increase in both risperidone and 9-hydroxyrisperidone plasma concentrations. The fraction absorbed in the verapamil group was 3.18 times higher than in the oral group (65.9% and 20.7%

for POV group and PO group). The absorption rate constant was lower in the verapamil group. Their results indicated that P-gp decreased the intestinal absorption of risperidone. The absence of 9-hydroxyrisperidone in experimental conditions led to exclude any involvement of CYP3A4 from the variations observed in the risperidone absorption step. The difference observed between absorption rate constants (0.28, 0.53 for PO and POV group, respectively) indicated that the absorption step was longer without verapamil co-administration. AUC and C_{max} are significantly increased for both risperidone and 9-hydroxyrisperidone. Ketoconazole, fenofibrate and erythromycin, all P-gp inhibitors, (Takano *et al.*, 1998 and Ehrhardt *et al.*, 2004), were tested. They increased risperidone absorption at a factor of 2.13, 2.19 and 1.40, respectively. The results of the *in vitro* Caco-2/TC7 study clearly indicated that P-gp is involved in the intestinal absorption of risperidone. Some more experiments are needed to evaluate potential interactions between risperidone and inhibitors or inducers of P-gp. In this Caco-2 study, CYP 3A4 does not seem to be implicated in cell metabolism owing to the absence of formation of 9-hydroxyrisperidone. In the animal study, they have also demonstrated that the luminal CYP3A4 does not affect the absorption of risperidone (Schmiedlin-Ren *et al.*, 2001). An evaluation of the risperidone plasma level seems to be the best solution to control its efficacy. Until results of further experiments on humans are available, physicians and pharmacists should beware of drugs altering P-gp activity which may also cause clinical changes in risperidone plasma concentrations (Etienne *et al.*, 2007). In our study, ketoconazole could inhibit hepatic CYP3A4 and lead to increasing of the AUC of risperidone and decreasing of 9-hydroxyrisperidone. While C_{max} and T_{max} of risperidone and 9-hydroxyrisperidone were not changed because ketoconazole (200 mg) has minimal effect on the absorptive phase as intestinal CYP3A4 was not significantly affected (Etienne *et al.*, 2007).

In our study, the Cl/f of risperidone was significantly decreased by 39.82% and Cl/f of 9-hydroxyrisperidone was significantly increased by 117.32% (Table 24). Therefore, the AUC of risperidone was significantly increased by 53.7% while AUC of 9-hydroxyrisperidone was significantly decreased by 49.95%. Therefore, ketoconazole should have some inhibitory effect on the metabolism of risperidone, especially on CYP3A4. Our finding is in accordance with other studies such as Glynn *et al*, (1986) who studied the effects of ketoconazole on methylprednisolone. The methylprednisolone dose was reduced by 57% when ketoconazole was administered chronically for 1 week to seek equivalent methylprednisolone AUCs by compensating for the expected reduction in methylprednisolone clearance. Ketoconazole decreased clearance of methylprednisolone by 46% and increased mean residence time by 37%. Based on the reduction in methylprednisolone clearance by ketoconazole, a 50% lower dose of methylprednisolone during concomitant therapy with ketoconazole is recommended. Fleishaker *et al*, (2003) reported the interaction between almotriptan and the potent CYP3A4 inhibitor ketoconazole. Ketoconazole coadministration increased mean almotriptan AUC and C_{max} from 312 to 490 ng hr/ml and 52.6 to 84.5 ng/ml, respectively. An accompanying increase in the fraction of almotriptan excreted unchanged in the urine (40.6% to 53.3%) and a decrease in renal clearance (16.4 to 13.8 l/hr). These effects were statistically significant. The effects of ketoconazole on

almotriptan clearance were consistent with inhibition of the CYP3A4-mediated metabolism and a slight effect on the active tubular secretion of almotriptan.

In this study, the $T_{1/2}$ of risperidone was significantly increased by 32.8% (Table 24) because ketoconazole inhibit metabolism of this compound. Kovarik. et al. (2005) sought to quantify the influence of the CYP3A and P-glycoprotein inhibitor i.e. ketoconazole on the pharmacokinetics of everolimus in healthy subjects. During ketoconazole coadministration, everolimus half-life was prolonged by 1.9-fold from 30 ± 4 hours to 56 ± 5 hours, the maximum concentration increased 3.9-fold (90% confidence interval, 3.4-4.6) from 15 ± 4 ng/ml to 59 ± 13 ng/ml. Everolimus area under the curve increased 15.0-fold (90% confidence interval, 13.6-16.6) from 90 ± 23 ng.h/ml to 1324 ± 232 ng.h/ml.

The pharmacokinetic parameter of risperidone (i.e. C_{max}, T_{max}) and 9-hydroxyrisperidone (i.e. C_{max}, $T_{max,}$, $T_{1/2}$, Vd/f) were not significantly changed after pretreatment with ketoconazole (Table 24). Our results were similar to the previous research (Mohamed et al., 2004) which showed that pretreatment with ketoconazole did not significantly increased azimilide C_{max} (12%), AUC (16%) and $T_{1/2}$ (13%). No change in T_{max}, Vd/f or plasma protein binding was observed. Inhibition appears to be mediated systemically since azimilide is completely absorbed following oral administration and increase in the terminal exponential half-life (12%) was observed upon coadministration with ketoconazole, with no change in the volume of distribution. The increase in azimilide concentration was small and was not considered to be clinically important. In addition, the pharmacologically active metabolites of azimilide are primarily formed via CYP3A4. Thus, CYP3A4 inhibition would decrease their formation and their corresponding plasma concentrations (normally about 10% or less of that of azimilide), reducing further any minimal contribution to the overall cardiovascular response associated with azimilide administration. Azimilide coadministration of ketoconazole caused a 14% decrease in azimilide total clearance. No clinically important drug interactions are expected when azimilide dihydrochloride is coadministered with CYP3A4 inhibitors.

Vermeir et al. (2008) reported, one week following administration of a single oral dose of 9-hydroxyrisperidone 1 mg, 59% of the dose was excreted unchanged into urine, indicating that 9-hydroxyrisperidone is not extensively metabolised by the liver. Approximately 80% of the administered radioactivity was recovered in urine and 11% in the faeces. Four metabolic pathways, i.e. dealkylation, hydroxylation, dehydrogenation, and benzisoxazole scission, have been identified in vivo, none of which accounted for more than 6.5% of the dose. Although in vitro studies suggested a role for CYP2D6 and CYP3A4 in the metabolism of 9-hydroxyrisperidone, there is no evidence in vivo that these isozymes play a significant role in the metabolism of 9-hydroxyrisperidone. Our results showed that the C_{max}, T_{max}, $T_{1/2}$ and Vd/f of 9-hydroxyrisperidone were not significantly changed because ketoconazole had no effect on either absorption or elimination of 9-hydroxyrisperidone. In vitro studied in human liver microsomes showed that 9-hydroxyrisperidone does not substantially inhibit the metabolism of drugs metabolised by CYP1A2, CYP2A6, CYP2C8/9/10, CYP2D6, CYP2E1, CYP3A4, and CYP3A5 (Vermeir et al., 2008).

Although elimination of risperidone after pretreatment with ketoconazole was statistical significantly reduced which led to significantly increase in half life, AUC_{0-48} and AUC_{0-96} of risperidone but the volunteers were still able to tolerate with such the changes without any toxic effect.

In conclusion, the results of this study have suggested that 3 days pretreatment with 200 mg of oral ketoconazole causes a significantly increase in AUC and $T_{1/2}$ of a single oral dose of 2 mg risperidone. Thus, clinicians should consider monitoring of the dose of risperidone in the patient who is concomitantly taking ketoconazole especially in the patient who received high dose of risperidone or in the elderly, renal or liver impairment patients who may have to reduce the dose of risperidone. Anyway, although plasma level of risperidone is increased but the active metabolite, 9-hydroxyrisperidone is decreased. The therapeutic outcome may not be changed in the early phase of treatment. Therefore, these patients should be closely monitored throughout the course of treatment.

REFERENCES

Abdel-Rahman, S.M., Gotschall, R.R., Kauffman, R.E., *et al.* 1999. Investigation of terbinafine as a CYP2D6 inhibitor *in vivo. Clin Pharmacol Ther.*; 65:465-72.

Abraham, M.A, Thomas, P.P, John, G.T., *et al.* 2003. Efficacy and safty of low-dose ketoconazole (50 mg) to reduce the cost of cyclosporine in renal allograft recipients. *Transplantation Proceedings.*; 35:215-216.

Allain, H., Tessier, C., Bentué-Ferrer, D., Tarral, A. *et al.* 2003. Effects of risperidone on psychometric and cognitive functions in healthy elderly volunteers. *Psychopharmacol. (Berl).*; 165(4):419-29.

Aman, M.G., Vinks, A.A., Remmerie, B., *et al.* 2007. Plasma pharmacokinetic characteristics of risperidone and their relationship to saliva concentrations in children with psychiatric or neurodevelopmental disorders. *Clin Ther.*; 29(7):1476-86.

Andreasen, N.C., Flaum, M., Swayze, V.W. 2nd *et al.* 1990. Positive and negative symptoms in schizophrenia. A critical reappraisal. *Arch Gen Psychiatry*; 47(7):615-21.

Annas, A., Carlstrom, K., Alov'an, G., and AL-Shurbaji, A. 2003. The effect of ketoconazole and diltiazem on oestrogen metabolism in postmenopausal women after single dose oestradiol treatment. *Br. J. Clin . Pharmacol.;* 56:334-336.

Anon. 1992. Drugs for treatment of fungal infection. *Med. Lett. Drugs Ther.*; 34:14-16.

Anon. 1993. Report of dangerous cardiac arrythmias prompt new contraindications for the drug Hismanal. *FDA Med. Bull.*; 23(1):2-3.

Anzenbacher, P., Anzenbacherova, E. 2001. Cytochromes P450 and metabolism of xenobiotics. *Cell Mol Life Sci.*; 58:737-47.

Arman, S., Sadramely, M.R., Nadi, M., *et al.* 2008. A randomized, double-blind, placebo-controlled trial of metformin treatment for weight gain associated with initiation of risperidone in children and adolescents. *Saudi Med J.*; 29(8):1130-4.

Auclair, A.L., Kleven, M.S., Barret-Grévoz, C., *et al.* 2008. Differences among conventional, atypical and novel putative D (2)/5-HT (1A) antipsychotics on catalepsy-associated behaviour in cynomolgus monkeys. *Behav Brain Res.*; 203(2):288-95.

Ayrton, A., Morgan, P. 2001. Role of transport proteins in drug absorption, distribution and excretion. *Xenobiotica.;* 31:469-97.

Backman, J.T., Granfors, M.T., Neuvonen, P.J. 2006. Rifampicin is only a weak inducer of CYP1A2-mediated presystemic and systemic metabolism: studies with tizanidine and caffeine. *Eur J Clin Pharmacol.;* 62:451-61.

Backman, J.T., Kyrklund, C., Neuvonen, M., Neuvonen, P.J. 2002. Gemfibrozil greatly increases plasma concentrations of cerivastatin. *Clin Pharmacol Ther.;* 2:685-91.

Backman, J.T., Olkkola, K.T., Neuvonen, P.J. 1996a Rifampin drastically reduces plasma concentrations and eff ects of oral midazolam. *Clin Pharmacol Ther,;* 59:7-13.

Backman, J.T., Olkkola, K.T., Ojala, M., Laaksovirta, H., Neuvonen, P.J. 1996b. Concentrations and effects of oral midazolam are greatly reduced in patients treated with carbamazepine or phenytoin. *Epilepsia.;* 37:253-7.

Balbi, C., D'Ajello, M., and Balbi, G.C. 1986. Treatment with ketoconazole in diabetic patients with vaginal candidiasis. *Drugs Exp. Clin. Res.;* 12: 413-414.

Baldwin, S.J., Bloomer, J.C., Smith, G.I., Ayrton, A.D., and Clarke, S.E. 1995. Ketoconazole and sulphaphenazole as the respective selective inhibitors of P450 3A and 2C9. *Xenobiotica.;* 25:2610-271.

Bersani, G., Quartini, A., Piperopoulos, O., *et al.* 2006. Brain abnormalities in schizophrenia. A qualitative comparative study of schizophrenic patients and control individuals assessed by magnetic resonance imaging. *J Neuroradiol.;* 33(3):152-7.

Bertz, R.J., Granneman, G.R. 1997. Use of *in vitro* and *in vivo* data to estimate the likelihood of metabolic pharmacokinetic interactions. *Clin Pharmacokinet.;* 32:210-58.

Bhaskar, C., Das', Ankanahlli, V., Madhukumar, Jaime Anguiano. 2008. Synthesis of novel ketoconazole derivatives as inhibitors of the human Pregnane X Receptor (PXR; NR1I2; also termed SXR, PAR). *Bioorg Med Chem Lett.;* 15; 18(14):3974-7.

Bostwick, J.R., Guthrie, S.K., Ellingrod, V.L. 2009. Antipsychotic-induced hyperprolactinemia. *Pharmacotherapy;* 29(1):64-73.

Boulton, D.W., DeVane, C.L., Liston, H.L., *et al.* 2002. *In vitro* P-glycoprotein affinity for atypical and conventional antipsychotics, *Life Sci.;* 71:163–169.

Brass, C., Galgiani, J.N., Blaschke, T.F., *et al*. 1982. Disposition of ketoconazole, an oral antifungal, in humans. *Antimicrob. Agents Chemother.* 21:151-158.

Brown, M.W., Maldonado, A.L., Meredith, C.G., *et al*. 1985. Effect of ketoconazole on hepatic oxidative drug metabolism. *Clin. Pharmacol Ther.;* 37:290-297.

Bruins, Slot L.A., Bardin, L., Auclair, A.L., *et al*. 2008. Effects of antipsychotics and reference monoaminergic ligands on marble burying behavior in mice. *Behav Pharmacol.*; 19(2):145-52.

Carlson, J.A., Mann, H.J., and Canafax, D.M. 1983. Effect of pH on disintegration and dissolution of ketoconazole tablets. *Am. J. Hosp. Pharm.;* 40:1334-1336.

Chambers, H.F. 2001. Antimicrobial Agents. Goodman's: The pharmacological Basis of Therapeutics (10th ed.). New York: *McGraw- Hall* Interanational Inc. pp. 770-779

Cleary, JD, Taylor, JW, Chapman, SW. 1992. Itraconazole in antifungal therapy. *Ann Pharmacother.*; 26(4):502-9.

Como, J.A., and Dismukes, W.E. 1994. Oral azole drugs as systemic antifungal therapy. *New Eng. J. Med.*; 330:263-272.

Cope, M.B., Li, X., Jumbo-Lucioni, P., DiCostanzo, C.A., *et al*. 2008. Risperidone alters food intake, core body temperature, and locomotor activity in mice. *Physiol Behav.*; 96(3):457-63.

Cordoba-Diaz, D., Cordoba-Diaz, M., Awad, S., and Cordoba-Borrego, M. 2001. Effect of pharmacotechnical design on *in vitro* interaction of ketoconazole tablet with non systemic antacid. *Int. J. Pharm.*; 226:61-68.

Court, M.H., Duan, S.X., Hesse, L.M., Venkatakrishnan, K., Greenblatt, D.J. 2001. Cytochrome P-450 2B6 is responsible for interindividual variability of propofol hydroxylation by human liver microsomes. *Anesthesiology*; 94:110-9.

Cousein, E., Barthélémy, C., Poullain, S., *et al*. 2007. P-glycoprotein and cytochrome P450 3A4 involvement in risperidone transport using an *in vitro* Caco-2/TC7 model and an *in vivo* model. *Prog Neuropsychopharmacol Biol Psychiatry.*; 31(4):878.

Cribb, A.E., Peyrou, M., Muruganandan, S., Schneider, L. 2005. The endoplasmic reticulum in xenobiotic toxicity. *Drug Metab Rev.*37:405-42.

Daly, A.K. 2003. Pharmacogenetics of the major polymorphic metabolizing enzymes. *Fundam Clin Pharmacol.*; 17:27-41.

Daly, A.K. 2006. Significance of the minor cytochrome P450 3A isoforms. *Clin Pharmacokinet.*; 45:13-31.

Daneshmend, T.K. 1990. Diseases and drugs but not food decrease ketoconazole bioavailability. *Br J Clin Pharmaco.*; 29:783-784.

Daneshmend, T.K., Mason, A.R., Lowe, C.H., *et al.* 1986. Influence of formulation on ketoconazole pharmacokinetics in man: comparison of standard tablet versus capsule containing citric acid. *J Antimicrob Chemother.*; 18(2):289-91.

Daneshmend, T.K., Mason, A.R., Warnock, D.W., *et al.* 1984. Influence of food on the pharmacokinetics of ketoconazole. *Antimicrob. Agents. Chemother.*; 25(1):1-3

Daneshmend, T.K., Warnock, D.W., Ene, M.D.. *et al.* 1983. Multiple dose pharmacokinetics of ketoconazole and their effects on antipyrine kinetic in man. *Antimicrob. Agents. Chemother.*; 226:61-68.

de Bruijn, P., Kehrer, D.F., Verweij, J., *et al.* 2001. Liquid chromatographic determination of ketoconazole, a potent inhibitor of CYP3A4-mediated metabolism. *J Chromatogr B Biomed Sci Appl.*; 753(2):395-400.

de Leon, J., Sandson, N.B., and Cozza, K.L. 2008. A preliminary attempt to personalize risperidone dosing using drug-drug interactions and genetics: part I. Psychosomatics.; 49(3):258-70.

Demling, J.H., Huang, M.L., Remmerie, B., *et al.* 2006. Pharmacokinetics and safety of combination therapy with lithium and risperidone. *Pharmacopsychiatry*; 39(6):230-1.

Dervaux, A., Levasseur, M. 2008. Risperidone and valproate for mania following stroke. *J Neuropsychiatry Clin Neurosci.*; 20(2):247.

Desta, Z., Zhao, X., Shin, J.G., Flockhart, D.A. 2002. Clinical signifi cance of the cytochrome P450 2C19 genetic polymorphism. *Clin Pharmacokinet.*, 41:913-58.

DeVane, C.L., Nemeroff, C.B. 2001. An evaluation of risperidone drug interactions. *J Clin Psychopharmacol.*; 21(4):408-16.

Ding, X., Kaminsky, L.S. 2003. Human extrahepatic cytochromes P450: function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. *Annu Rev Pharmacol Toxicol.*; 43:149-73.

Drake, R.E., Mueser, K.T., Torrey, W.C., *et al*. 2000. Evidence-based treatment of schizophrenia. *Curr Psychiatry Rep.*; 2(5):393-7.

Dresser, G.K., Spence, J.D., Bailey, D.G. 2000. Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. *Clin Pharmacokinet.*; 38:41-57.

DuBuske, L.M. 2005. The role of P-glycoprotein and organic anion-transporting polypeptides in drug interactions. *Drug Safty;* 28:789-801.

Duggal, H.S., Mendhekar, D.N. 2008. Risperidone-induced tardive pharyngeal dystonia presenting with persistent Dysphagia: a case report. *Prim Care Companion J Clin Psychiatry*; 10(2):161-2.

Durr, D., Stieger, B., Kullak-Ublick, G.A., Rentsch, K.M., *et al*. 2000. St John's Wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. *ClinPharmacol Ther.*; 68:598-604.

Eagling, V.A., Tjia, J.F., Back, D.J. 1998. Differential selectivity of cytochrome P450 inhibitors against probe substrates in human and rat liver microsomes. *Br J Clin Pharmacol.*; 45:107-14.

Eckland, D.A., Danhof, M. 2000. Clinical pharmacokinetics of pioglitazone. *Exp Clin Endocrinol Diabetes.*; 108 Suppl. 2:234-42.

Ehrhardt, M., Lindenmaier, H., Burhenne, J., *et al*. 2004. Influence of lipid lowering fibrates on P-glycoprotein activity *in vitro*. *Biochem Pharmacol.*; 67, (2), 285–292.

Eichenbaum, G., Pollock-Dove, C., Nguyen ,J., *et al*. 2006. Preclinical assessment of the feasibility of applying controlled release oral drug delivery to a lead series of atypical antipsychotics. *J Pharm Sci.*; 95(4):883-95.

Ekins, S., Wrighton, S.A. 1999. The role of CYP2B6 in human xenobiotic metabolism. *Drug Metab Rev.*; 31:719-54.

Enayetallah, A.E., French, R.A., Thibodeau, M.S., Grant, D.F. 2004. Distribution of soluble epoxide hydrolase and of cytochrome P450 2C8, 2C9, and 2J2 in human tissues. *J Histochem Cytochem.*; 52:447-54.

Etienne, C., Christine, B., Stéphanie, P., *et al*. 2007. P-glycoprotein and cytochrome P450 3A4 involvement in risperidone transport using an *in vitro* Caco-2/TC7 model and an *in vivo* model. *Progress in Neuro-Psychopharmacology and Biological Psychiatry.*; 31(4):878-886.

Fabris, A., Pellanda, M.V., Gardin, C., *et al.* 1993. Pharmacokinetics of antifungal agents. *Perit Dial Int.*; 13 Suppl. 2:S380-2.

Fauci, A.S., Macher, A.M., Lango, D.L., *et al.* 1984. Acquired immunodeficiency syndrome: *Ann. Intern. Med.*; 100:92-106.

Fenneteau, F., Poulin, P., Nekka, F. 2009. Physiologically based predictions of the impact of inhibition of intestinal and hepatic metabolism on human pharmacokinetics of CYP3A substrates. *J Pharm Sci; 28.* [Epub ahead of print].

Fisman, S., Reniers, D., Diaz, P. 1996. Erythromycin interaction with risperidone or clomipramine in an adolescent. *J Child Adolesc Psychopharmacol.*; 6(2):133-8.

Fleishaker, J.C., Herman, B.D., Carel, B.J., *et al.* 2003. Interaction between ketoconazole and almotriptan in healthy volunteers. *J Clin Pharmacol.*; 43(4):423-7.

Fleishaker, J.C., Pearson, P.G., Wienkers, L.C., *et al.* 1996. Biotransformation of tirilazad in human: 2. Effect of ketoconazole on tirilazad clearance and oral bioavailability. *J. Pharmacol. Exp. Ther.*; 227(2):991-998.

Fontana, E., Dansette, P.M., Poli, S.M. 2005. Cytochrome p450 enzymes mechanism based inhibitors: common sub-structures and reactivity. *Curr Drug Metab.*; 6:413-54.

Fricker, G., Drewe, J., Huwyler J., *et al.* 1996. Relevance of p-glycoprotein for the enteral absorption of cyclosporin A: *in vitro–in vivo* correlation. *Br J Pharmacol.*; 118: 841–1847.

Fromm, M.F. 2003. Importance of P-glycoprotein for drug disposition in humans. *Eur J Clin Invest.*; 33 Suppl. 2:6-9.

Fuhr, U. 2000. Induction of drug metabolising enzymes: pharmacokinetic and toxicological consequences in humans. *Clin Pharmacokinet.*; 38:493-504.

Gan, L.S.L., Moseley, M.A., Khosla, B., *et al.* 1996 CYP3A-Like cytochrome P450-mediated metabolism and polarized efflux of cyclosporin A in Caco-2 cells: interaction between the two biochemical barriers to intestinal transport, *Drug Metab Dispos.*; 24: 344–349.

Gascoigne, E.W., Barton, G.J., Michaels, M., *et al.* 1981. The kinetics of ketoconazole in animal and man. *Clin. Res.* (Review); 1: 177-187.

Gerbal-Chaloin S., Pascussi J.M., Pichard-Garcia L., *et al.* 2001. Induction of CYP2C genes in human hepatocytes in primary culture. *Drug Metab Dispos.*; 29:242-51.

Giacomini, K.M., Sugiyama, Y. 2006. Membrane transporters and drug response. In: Brunton, L.L., Lazo, J.S., Parker K.L., editors. *Goodman & Gilman's the pharmacological basis of therapeutics*. New York: McGraw-Hill. pp. 41-70.

Ghetti, P., Partrone, P., and Tosti, A. 1986. Ketoconazole in the treatment of acne in women (letter). *Arch. Dermatol.*; 122: 629.

Gibson, G. G., Plant, N. J., Swales, K. E., *et al.* 2002. Receptor-dependenttranscriptional activation of cytochrome P4503A genes: induction mechanisms, species differences and interindividual variation in man. *Xenobiotica.*; 32(3):165-206.

Ginsburg C.M., McCracken G.H. Jr, Olsen K. 1983. Pharmacology of ketoconazole suspension in infants and children. *Antimicrob Agents Chemother.*; 23(5):787-9.

Giacomini K.M., Sugiyama Y. 2006. Membrane transporters and drug response. In: Brunton L.L., Lazo J.S., Parker K.L., editors. *Goodman & Gilman's the pharmacological basis of therapeutics*. New York: McGraw-Hill. pp. 41-70.

Ginsburg, C.M., McCracken, G.H., and Olsen, K. 1983. Pharmacology of ketoconazole suspension infants and children. *Antimicrob. Agents Chemother.*; 23:787-789.

Glynn, A.M., Slaughter, R.L., Brass, C., *et al.* 1986. Effects of ketoconazole on methylprednisolone pharmacokinetics and cortisol secretion. *Clinical pharmacology and therapeutics.*; 39(6):654-9.

Gotoh O. 1992. Substrate recognition sites in cytochrome P450 family 2 (CYP2) proteins inferred from comparative analyses of amino acid and coding nucleotide sequences. *J Biol Chem.*; 267:83-90.

Granfors M.T., Backman J.T., Laitila J., *et al.* 2004. Tizanidine is mainly metabolized by cytochrome P450 1A2 in vitro. *Br J Clin Pharmacol.*; 57:349-53.

Graybill J.R., Galgiani J.N., Jorgensen J.H., *et al.* 1983. Ketoconazole therapy for fungal urinary tract infections. *J Urol.*; 129(1):68-70.

Graybill, J.R., and Drutz, D.J. 1980. Ketoconazole: A major innovation for treatment of fungal decrease. *Ann. Intern. Med.;* 93:921-923.

Greenblatt, D.J., von Moltke, L.L., Harmatz, J.S., *et al.* 1993. Alplazolam pharmacokinetics, metabolism, and plasma levels: clinical implications. *J. Clin. Psychiatry.*; 54 (suppl 10):4-11.

Greenblatt, D.J., von Moltke, L.L., Harmatz, J.S., *et al.* 1998. Kinetic and dynamic interaction study of zolpidem with ketoconazole, itraconazole, and fluconazole. *Clin. Pharmacol. Ther.;* 64:661-671.

Guengerich, F.P., Brain, W.R., Iwasaki, M., *et al.* 1991. Oxidation of dihydropyridine calcium channel blockers and analogues by human liver cytochrome P450 3A4. *J. Med. Chem.;* 34: 1838-1844.

Gutierrez R., Lee P.I., Huang M.L., *et al.* 1997. Risperidone: effects of formulations on oral bioavailability. *Pharmacotherapy*; 17(3):599-605.

Handschin C., Meyer U.A. 2003. Induction of drug metabolism: the role of nuclear receptors. *Pharmacol Rev.;* 55:649-73.

Hardy T.A., Marquez E., Kryzhanovskaya L., *et al.* 2006. Cross-sectional comparison of fasting lipids in normoglycemic patients with schizophrenia during chronic treatment with olanzapine, risperidone, or typical antipsychotics. *J Clin Psychopharmacol.*; 26(4):405-8.

Heel, R.C., Brogden, R.N., Carmine, A., *et al.* 1982. Ketoconazole : a review of its therapeutic efficacy in superficial and systemic fungal infections. *Drugs;* 231-236.

Heinig, R., Adelmann, H.F., and Ahr, G. 1999. The effect of ketoconazole on the pharmacokinetics, pharmacodynamics and safety of nisoldipine. *Eur. J. Clin. Pharmacol.*; 55:57-60.

Herman, B.D., Fleishaker, J.C., and Brow, M.T. 1999. Ketoconazole inhibits the clearance of the enantiomers of antidepressant reboxetine in humans. *Clin. Pharmacol. Ther.;* 66(4):374-379.

Hesse, L.M., Venkatakrishnan K., Court, M.H., *et al.* 2000. CYP2B6 mediates the in vitro hydroxylation of bupropion: potential drug interactions with other antidepressants. *Drug Metab Dispos.*; 28:1176-83.

Heykants, J., Huang, M.L., Mannens G., *et al.* 1994. The pharmacokinetics of risperidone in humans: a summary. *Clin Psychiatry*; 55 (Suppl): 13-7.

Higashi, M.K., Veenstra, D.L., Kondo, L.M., Wittkowsky, A.K., Srinouanprachanh, S.L., Farin, F., Rettie, A.E. 2002. Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *Jama*; 287:1690-8.

Ho, R.H., Kim, R.B. 2005. Transporters and drug therapy: implications for drug disposition and disease. *Clin Pharmacol Ther.;* 78:260-77.

Hollenberg, P.F. 2002. Characteristics and common properties of inhibitors, inducers, and activators of CYP enzymes. *Drug Metab Rev.*; 34:17-35.

Honkakoski, P., Sueyoshi, T., Negishi, M. 2003. Drug-activated nuclear receptors CAR and PXR. *Ann Med.;* 35:172-82.

Hoppu, K., Tapanainen, P., Tikanoja, T., *et al.* 1991. Accidental astemizole overdose in yong children. *Lancet.;* 338:538-539.

Horrigan, J.P, Barnhill, L.J., *et al.* 1997. Risperidone and explosive aggressive autism. *J Autism Dev Disord.*; 27(3):313-23.

Huang, M.L., Van, P.A., Woestenborghs R., *et al.* 1993. Pharmacokinetics of the novel antipsychotic agent risperidone and the prolactin response in healthy subjects. *Clin Pharmacol Ther.*; 54(3):257-68.

Ingelman-Sundberg M. 2004. Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future. *Trends Pharmacol Sci.*; 25:193-200.

Ingelman-Sundberg, M. 2005. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J.*; 5:6-13.

Ingelman-Sundberg, M., Oscarson, M., Daly. A.K., *et al.* 2001. Human cytochrome P-450 (CYP) genes: a web page for the nomenclature of alleles. *Cancer Epidemiol Biomarkers Prev.*; 10:1307-8.

Isbister, G.K., and Whyte, I.M. 2002. Atypical presentation of risperidone toxicity. *Vet Hum Toxicol.*; 44(2):118-9.

Ito, K., Iwatsubo, T., Kanamitsu. S., *et al.* 1998a. Quantitative prediction of *in vivo* drug clearance and drug interactions from *in vitro* data on metabolism, together with binding and transport. *Annu Rev Pharmacol Toxicol.*; 38:461–99.

Ito, K., Iwatsubo, T., Kanamitsu, S., *et al*, 1998b. Prediction of pharmacokinetic alterations caused by drug-drug interactions: metabolic interaction in the liver. *Pharmacol Rev.*; 50: 387–411.

Ito, K., Kusuhara, H., Sugiyama, Y. 1999. Effects of intestinal CYP3A4 and P glycoprotein on oral drug absorption-theoretical approach. *Pharm Res*; 16:225–231.

Janssen, P.A.J., Niemegeer C.J.E., Awouters F., *et al.*, 1988. Pharmacology of risperidone, a new antipsychotic with serotonin-S_2 and dopamine-D_2 antagonistic properties. *J. Pharmacol Exp Ther.;* 244:685-693.

Josefsson, M., Zackrisson, A.L., and Ahlner, J. 1996. Effect of grapefruit juice on the pharmacokinetics of amlodipine in healthy volunteers. *Eur. J. Clin. Pharmacol.;* 51:189-193.

Kapur, S., Langlois, X., Vinken, P., Megens, A.A., *et al.* 2002. The differential effects of atypical antipsychotics on prolactin elevation are explained by their differential blood-brain disposition: a pharmacological analysis in rats. *J Pharmacol Exp Ther.*; 302: 1129-1134.

Kent, U.M., Juschyshyn, M.I., Hollenberg, P.F. 2001. Mechanism-based inactivators as probes of cytochrome P450 structure and function. *Curr Drug Meta.;* 2:215-43.

Keogh, J.P., Kunta, J.R. 2006. Development, validation and utility of an in vitro technique for assessment of potential clinical drug-drug interactions involving P-glycoprotein. Eur J Pharm Sci.; 27(5):543-54.

Khaliq, Y., Gallicano, K., Venance, S., *et al.* 2000. Effect of ketoconazole on ritonavir and saquinavir concentrations in plasma and cerebrospinal fluid from patients infected with human immunodeficiency virus. *Clin Pharmacol. Ther.*; 68:637-646.

Kim, A.E., Dintaman, J.M., Waddell, D.S., *et al.* 1998. Saquinavir, an HIV protease inhibitor, is transported by P-glycoprotein. *J Pharmacol Exp Ther.*; 286(3):1439-45.

Kim, R.B. 2003. Organic anion-transporting polypeptide (OATP) transporter family and drug disposition. *Eur J Clin Invest.;* 33 Suppl. 2:1-5.

Kim, R.B., Fromm, M.F., Wandel *et al.* 1998. The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors, *J Clin Invest.*; 101: 289–294.

Kirchheiner, J., Brockmoller,, J. 2005. Clinical consequences of cytochrome P450 2C9 polymorphisms. *Clin Pharmacol Ther.*; 77:1-16.

Kirchheiner, J., Roots, I., Goldammer, M., *et al.* 2005. Effect of genetic polymorphisms in cytochrome P450 (CYP) 2C9 and CYP2C8 on the pharmacokinetics of oral antidiabetic drugs: clinical relevance. *Clin Pharmacokinet.*; 44:1209-25.

Klein, K., Saussele, T., Toscano, C., Blievernicht, J., Eichelbaum M., Schwab M., Zanger U.M., 2006. Comprehensive analysis of drug metabolizing cytochromes P450 in human liver. In: *MDO. Book of Abstracts.*; Budabest. p.127.

Klingenberg, M. 1958. Pigments of rat liver microsomes. *Arch Biochem Biophys.*; 75:376-86.

Klotz, U., Schwab, M., and Treiber, G. 2004. CYP2C19 polymorphism and proton pump inhibitors. *Basic Clin Pharmacol Toxicol.*; 95:2-8.

Ko, J.W., Sukhova, N., Thacker, D., Chen, P., Flockhart, D.A. 1997. Evaluation of omeprazole and lansoprazole as inhibitors of cytochrome P450 isoforms. *Drug Metab Dispos*; 25:853-62.

Koch, H. 1983. Ketoconazole. *Pharm. Int.* 4:151-152.

Kohler, G.I., Bode-Boger, S.M., Busse, R., Hoopmann, M., Welte, T., Boger, R.H. 2000. Drug-drug interactions in medical patients: effects of in-hospital treatment and relation to multiple drug use. *Int J Clin Pharmacol Ther.*; 38:504-13.

Konig, J., Seithel, A., Gradhand, U., Fromm, M.F. 2006. Pharmacogenomics of human OATP transporters. *Naunyn Schmiedebergs Arch Pharmacol.;* 372:432-43.

Kosten, T.R., Oliveto, A., Sevarino, K.A., *et al.* 2002. Ketoconazole increses cocaine and opiod use in methadone maintained patients. *Drug and Alcohol Dependence.;* 66: 173-180.

Kovacs, G.T., Westcott, M., and Rusden, J. 1990. A prospective study to assess the efficacy of ketoconazole in the treatment of recurrent vaginal candidiasis. *Med. J. Aust.*; 153: 328-330.

Kovarik, J.M., Beyer, D., *et al.* 2005. Blood concentrations of everolimus are markedly increased by ketoconazole. *J Clin Pharmacol.*; 45(5):514-8.

Krishna, D.R., Klotz, U. 1994. Extrahepatic metabolism of drugs in humans. *Clin Pharmacokinet.*; 26:144-60.

Krishnaiah, Y.S.R., Satyanarayana, S., and Visweswarani, D. 1993. Drug interaction of tolbutamide with ketoconazole in diabetic rabbits. *Indian J. Pharmacol.*; 25(3):146-148.

Kuroha, M., Shirai, Y., Shimoda, M. 2004. Multiple oral dosing of ketoconazole influences pharmacokinetics of quinidine after intravenous and oral administration in beagle dogs. *J Vet Pharmacol Ther.*; 27(5):355-359.

Lamba, J.K., Lin, Y.S., Schuetz, E.G, Thummel KE. 2002. Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev.*; 54:1271-94.

Lane, H.Y., Chang, W.H. 1998. Risperidone–carbamazepine interactions; is cytochrome P450 3A involved. *J Clin Psychiatry*; 59(8):430-1.

Lang, T., Klein, K., Fischer, J., *et al.* 2001. Extensive genetic polymorphism in the human CYP2B6 gene with impact on expression and function in human liver. *Pharmacogenetics*; 11:399-415.

Lelawongs, P., Barone, J.A., Colaizzi, J.L., *et al.* 1988. Effect of food and gastric acidity on absorption of orally administered ketoconazole. *Clin Pharm.*; 7(3):228-35.

Leu, B.L. and Huang, J.D. 1995. Inhibition of intestinal P-glycoprotein and effects on etoposide absorption, *Cancer Chemother Pharmacol.*; 35 (1995), 432–436.

Lewis, D.F. 2004. Varieties: the human cytochromes P450. *Pharmacogenomics*; 5:305-18.

Lewis, D.F. 2000. Modelling human cytochromes P450 for evaluating drug metabolism: an update. *Drug Metabol Drug Interact.*; 16:307-24.

Leysen, J.E., Gommeren, W., Eens, A., *et al.* 1988. Biochemical profile of risperidone, a new antipsychotic. *J Pharmacol Exp Ther.*; 247(2):661-70.

Li, C., Xia, J., Wang, J. 2009. Risperidone dose for schizophrenia. *Cochrane Database Syst Rev.*; 7; (4):CD007474. DOI: 10.1002/14651858.

Lim, Y.P., Kuo, S.C., Lai, M.L., *et al.* 2009. Inhibition of CYP3A4 expression by ketoconazole is mediated by the disruption of pregnane X receptor, steroid receptor coactivator-1, and hepatocyte nuclear factor 4alpha interaction. *Pharmacogenet Genomics*; 19(1):11-24.

Lin, J.H., Lu, A.Y. 1998. Inhibition and induction of cytochrome P450 and the clinical implications. *Clin Pharmacokinet.* 35:361-90.

Lin, J.H. 2006. Tissue distribution and pharmacodynamics: a complicated relationship. *Curr Drug Metab.*; 7:39-65.

Loboz, K.K., Gross, A.S., Williams, K.M., *et al.* 2006. Cytochrome P450 2B6 activity as measured by bupropion hydroxylation: effect of induction by rifampin and ethnicity. *Clin Pharmacol Ther.*; 80:75-84.

Lown, K.S., Mayo, R.R., Leichtman, A.B., *et al.* 1997. Role of intestinal glycoprotein (mdr-1) in interpatient variation in the oral bioavailability of cyclosporine. *Clin Pharmacol Ther.*; 62 (1997), 248–260.

Madan, A., Graham, R.A., Carroll, K.M., *et al.* 2003. Effects of prototypical microsomal enzyme inducers on cytochrome P450 expression in cultured human hepatocytes. *Drug Metab Dispos.*; 31:421-31.

Maksymink, A.W., Levine, H.B., and Bodey, G.P. 1982. Pharmacokinetics of ketoconazole in patients with neoplastic diseases. *Antimicrob. Agents Chemother.;* 22:43-46.

Malaiyandi, V., Sellers, E.M., Tyndale, R.F. 2005. Implications of CYP2A6 genetic variation for smoking behaviors and nicotine dependence. *Clin Pharmacol Ther.;* 77:145-58.

Mandell, G.I., Douglas, R.G., and Bennett, J.E. (eds). 1990. Principles and Practice of Infectious Diseases, 3rd ed. Churchill Livingstone, New York, NY.

Mannens, G., Huang, M.L., Meuldermans, W., *et al.* 1993. Absorption, metabolism and excretion of risperidone in humans. *Drug Metab Dispos.*; 21: 1134–1140.

Mannens, G., Meuldermans, W., Snoeck, E., *et al.* 1994. Plasma protein binding of risperidone and its distribution in blood. *Psychopharmacology(Berl)*; 114 (4):566-72.

Mansuy, D. 1998. The great diversity of reactions catalyzed by cytochromes P450. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol.*; 121:5-14.

Marder, S.R., Davis, J.M., Chouinard, G. 1997. The effects of risperidone on the five dimensions of scihzopharenia derived by factor analysis: Combined results of the North American Trials. *J. Clin. Psychiatry.;* 58(12):538-546.

Martínez-Jordá, R., Rodriguez-Sasiain, J.M., Suárez, E., *et al.* 1990. Serum binding of ketoconazole in health and disease. *Int J Clin Pharmacol Res.*; 10(5):271-6.

McGrawth, J., Murphy, G.M. 1991. The control of seborrhoeic dermatitis and dandruff by antipityrosporal drugs. *Drugs*; 41(2):178-84.

Meyer, U.A. 1996. Overview of enzymes of drug metabolism. *J Pharmacokinet Biopharm.*; 24:449-59.

Meyer, K.C., McManus, E.J., and Maki, D.G. 1993. Overwhelming pulmonary blastomycosis associated with the adult respiratory distress syndrome. *N. Eng. J. Med.;* 329: 1231-1236.

Meyer, U.A. 1996. Overview of enzymes of drug metabolism. *J Pharmacokinet Biopharm.*; 24:449-59.

Miners, J.O., Birkett, D.J. 1998. Cytochrome P450 2C9: an enzyme of major importance in human drug metabolism. *Br J Clin Pharmacol.*; 45:525-38.

Miners, J.O, McKinnon, R.A. 2000. CYP1A. In: Levy RH, Th ummel KE, Trager WF, Hansten PD, Eichelbaum M, editors. *Metabolic drug interactions.* Philadelphia: Lippincott Williams & Wilkins. pp. 61-73.

Mirghani, R.A., Hellgren, U., Westerberg, P.A., *et al.* 1999. The roles of cytochrome P450 3A4 and 1A2 in the 3-hydroxylation of quinine *in vivo. Clin. Pharmacol. Ther.;* 66(5):454-460.

Mohamed E.l. Mouelhi., Dan, J. Worley., Barbara, Kuzmak., *et al.* 2004. Influence of ketoconazole on azimilide pharmacokinetics in healthy subjects. *Br J Clin Pharmacol.* ; 58(6): 641–647.

Moody, D.E., Walsh, S.L., Rollins, D.E., Neff, J.A., and Huang, W. 2004. Ketoconazole, a cytochrome P450 3A4 inhibitor, markedly increases concentrations of levo-acetyl-∞-methadol in opioid-naïve individuals. *Clin. Pharmacol. Ther.;* 53:457-462.

Mouelhi, M.E., Worley, D.J., Kuzmak, B., *et al.* 2004. Influnce of ketoconazole on azimilide pharmacokinetics in healthy subjects. *Br. J. Clin. Pharmacol.;* 58(6):641-647.

Nakagami, N., Yasui-Furukori, M., Saito *et al.,* 2005. Effect of verapamil on pharmacokinetics and pharmacodynamics of risperidone: *in vivo* evidence of involvement of P-glycoprotein in risperidone disposition, *Clin Pharmacol Ther.*; 78(9)43–51.

Nebert, D.W., Russell, D.W. 2002. Clinical importance of the cytochromes P450. *Lancet*; 360:1155-62.

Nelson, D.R., Koymans, L., Kamataki, T., et al. 1996. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics*; 6:1-42.

Neuvonen, P.J., and Suhonen, R. 1995. Itraconazole interact with felodipine. *J. Am. Acad. Dermatol.;* 33:134-135.

Niemi, M., Backman, J.T., Granfors, M., et al. 2003b. Gemfibrozil considerably increases the plasma concentrations of rosiglitazone. *Diabetologia.;* 6:1319-23.

Niemi, M., Backman, J.T., Neuvonen, M., Neuvonen, P.J. 2003c. Effects of gemfibrozil, itraconazole, and their combination on the pharmacokinetics and pharmacodynamics of repaglinide: potentially hazardous interaction between gemfibrozil and repaglinide. *Diabetologia.*; 6:347-51.

Niemi, M., Backman, J.T., Neuvonen. P.J. 2004a. Effects of trimethoprim and rifampin on the pharmacokinetics of the cytochrome P450 2C8 substrate rosiglitazone. *Clin Pharmacol Ther.*; 6:239-49.

Nourian, Z., Mow, T., Muftic,D., Burek, S. et al., 2008. Orthostatic hypotensive effect of antipsychotic drugs in Wistar rats by *in vivo* and *in vitro* studies of alpha1-adrenoceptor function. *Psychopharmacology(Berl)*; 199(1):15-27.

Nyberg, S., Farde, L., Eriksson, L., Halldin, C., et al. 1993. 5-HT_2 and dopamine- D_2 receptor occupancy in the living human brain. *Psychopharmacol.,* 110:265-272.

Obach, R.S., Zhang, Q.Y., Dunbar, D., et al. 2001. Metabolic characterization of the major human small intestinal cytochrome p450s. *Drug Metab Dispos.*; 29:347-52.

Oftebro, H., Jensen, J., Mowinchel , P., et al., 1994. Establishing a ketoconazole suppression test for verifying testosterone administration in the doping control of athlets. *J. Clin. Endocrinol. Metab.;* 78(4):973-977.

Ogilvie, B.W., Zhang, D., Li, W., et al. 2006. Glucuronidation converts gemfi brozil to a potent, metabolism-dependent inhibitor of CYP2C8: implications for drug-drug interactions. *Drug Metab Dispos.*; 4:191-7.

Olkkola, K.T., Backman, J.T., and Neuvonen, P.J. 1994. Midazolam should be avoided in patients receiving the systemic antimycotics ketoconazole or itraconazole. *Clin Pharmacol Ther.*; 55: 481–485.

Omura. T., Sato, R. 1962. A new cytochrome in liver microsomes. *J Biol Chem.*; 237:1375-6.

Ong, C.E., Coulter, S., Birkett, D.J., Bhasker, C.R., Miners, J.O. 2000. The xenobiotic inhibitor profile of cytochrome P450 2C8. *Br J Clin Pharmacol.*; 50:573-80.

Ortiz de Montellano, P.R., Correia, M.A. 1995. Inhibition of cytochrome P450. In: Ortiz de Montellano, P.R., editor. *Cytochrome P450: structure, mechanism and biochemistry.* New York: Plenum Pr; pp. 305–66.

Osawa, Y., Pohl, L.R. 1989. Covalent bonding of the prosthetic heme to protein: a potential mechanism for suicide inactivation or activation of hemoproteins. *Chem Res Toxicol;* 2:131–41.

Owen, R.T. 2007. Extended-release paliperidone: efficacy, safety and tolerability profile of a new atypical antipsychotic. *Drugs Today (Barc)*; 43(4):249-58.

Park, J.Y., Kim, K.A., *et al.* 2004. Effect of ketoconazole on the pharmacokinetics of rosiglitazone in healthy subjects. *Br J Clin Pharmacol.*; 58(4):397-402.

Pelkonen, O. 2002. Human CYPs: *in vivo* and clinical aspects. *Drug Metab Rev.*; 34:37-46.

Pelkonen, O., Mäenpää, J., Taavitsainen, P., Rautio A., *et al.* 1998. Inhibition and induction of human cytochrome P450 (CYP) enzymes. *Xenobiotica.*; 28:1203-53.

Pelkonen, O., Rautio, A., Raunio, H., Pasanen, M. 2000. CYP2A6: a human coumarin 7-hydroxylase. *Toxicol.*; 144:139-47.

Pershing, L.K., Corlett, J., and Jorgensen, C. 1994. *In vivo* pharmacokinetics and pharmacodynamics of topical ketoconazole and miconazole in human statum cornuem. *Aniimicrob. Agents Chemother.;* 38(1):90-95.

Peterson, J.A., Graham, S.E. 1998. A close family resemblance: the importance of structure in understanding cytochromes P450. *Structure*; 6:1079-85.

Polk, R.E., Crouch, M.A., Israel, D.S., *et al.* 1999. Pharmacokinetic interaction between ketoconazole and amprenavir after single doses in healthy men. *Pharmacotherapy.;* 19:1378-1384.

Product Information. 1999. Abelcet, amphotericin B lipid complex. The Liposome Company, Inc., Princeton, NJ.

Product Information. 1996. Nizoral®, ketoconazole. Janssen Pharmaceutica, Titusville, NJ.

Product Information. 1998. Nizoral®, ketoconazole. Janssen Pharmaceutica, Titusville, NJ.

Product Information. 2000. Nizoral® 2% Shampoo. Janssen Pharmaceutica, Titusville, NJ.

Product Information. 1997. Versed®, midazolam. Roche Laboratories Inc., Titusville, NJ.

Raycy, J., Carpenter, S.P. 2000. CYP2E1. In: Levy RH, Th ummel KE, Trager WF, Hansten PD, Eichelbaum M, editors. *Metabolic drug interactions*. Philadelphia: Lippincott Williams & Wilkins. pp. 95-113.

Razaq, M., Samma, M. 2004. A case of risperidone-induced hypothermia. *Am J Ther.*; 11(3):229-30.

Remmerie, B.M., Sips, L.L., de Vries R., *et al.* 2003. *J Chromatogr B Analyt Technol Biomed Life Sci.*; 15; 783 (2):461-72.

Rendic, S. 2002. Summary of information on human CYP enzymes: human P450 metabolism data. *Drug Metab Rev.*; 34:83-448.

Rendic, S., Di, Carlo, F.J. 1997. Human cytochrome P450 enzymes: A status report summarizing their reactions, substrates, inducers, and Inhibitors. *Drug Metab Rev*; 29: 413-580.

Restrepo, A., Stevens, D.A., Gomez, I., *et al.* 1980. Ketoconazole: a new drug for the treatment of paracoccidiodomycosis. *Rev. Infect. Dis.;* 2:633-642.

Rettie, A.E., Jones, J.P. 2005. Clinical and toxicological relevance of CYP2C9: drug-drug interactions and pharmacogenetics. *Annu Rev Pharmacol Toxicol.* ; 45:477-94.

Richter, T., Murdter, T.E., Heinkele, G., *et al.* 2004. Potent mechanism-based inhibition of human CYP2B6 by clopidogrel and ticlopidine. *J PharmacolExp Ther.*; 308:189-97.

Riley, R.J. 2001. The potential pharmacological and toxicological impact of P450 screening. *Curr Opin Drug Discov Devel.*; 4:45-54.

Rosenberg, I., Aniskin, D., Bernay, L., *et al.* 2009. Psychiatric treatment of patients predisposed to priapism induced by quetapine, trazadone and risperidone: a case report. *Gen Hosp Psychiatry*; 31(1):98.

Rowland-Yeo, K., Rostami-Hodjegan, A., Tucker, G.T. 2004. Abundance of cytochromes P450 in human liver: a meta-analysis. *Br J Clin Pharmacol.*; 57:687-8.

Saadeddin, A., Torres-Molina, F., Carcel-Trullols, J., *et al.* 2004. Effect of cytochrome P450 inhibitors (diethyl dithiocarbamate, ketoconazole and grapefruit juice) on the pharmacokinetics of all-*trans*-retinoic acid. *ILFARMACO.*; 59:697-702.

Saito, M., Yasui-Furukori, N., Nakagami, T., *et al.* 2005. Dose-dependent interaction of paroxetine with risperidone in schizophrenic patients. *J Clin Psychopharmacol.*; 25(6):527-32.

Salphati, L., Benet, L.Z. 1998. Effects of ketoconazole on digoxin absorption and disposition in rat, *Pharmacology*; (56). 308–313.

Schmiedlin-Ren, P., Thummel, K.E., Fisher, J.M., *et al.* 2001. Induction of CYP3A4 by 1 alpha,25-dihydroxyvitamin D3 is human cell line-specific and is unlikely to involve pregnane X receptor, *Drug Metab Dispos.*; 29,1446–1453.

Schneider, R.A., Lizer, M.H. 2008. Apparent seizure and atrial fibrillation associated with paliperidone. *Am J Health Syst Pharm.*; 15; 65 (22):2122-5.

Serody, J.S., Mill, M.R., Detterbeck, F.C., *et al.* 1993. Blastomycosis in transpolant recipients: report of case and review. *Clin. Infect. Dis.;* 16:54-58.

Shimada, T., Yamazaki, H., Mimura, M., *et al.* 1994. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther.*; 270:414-23.

Shitara, Y., Sato, H., Sugiyama, Y. 2005. Evaluation of drug-drug interaction in the hepatobiliary and renal transport of drugs. *Annu Rev Pharmacol Toxicol.*; 45:689-723.

Silveira, L.H., Cuellar, M.L., Citera, G., *et al.* 1993. Candida arthritis. *Rheum. Dis. Clin. North. Am.*; 19: 427-437.

Silverman, R.B., George, C. 1998. Inactivation of gamma-aminobutyric acid aminotransferase by (Z)-4-amino-2-fluorobut-2-enoic acid. *Biochemistry*. 3; 27(9):3285-9.

Smith, A.G. 1984. Potentiation of oral anticoagulants by ketoconazole. *Br. Med. J.;* 288:188-189.

Snoeck, E., Van Peer A, Sack M., *et al.* 1995. Influence of age, renal and liver impairment on the pharmacokinetics of risperidone in man. *Psychopharmacology (Berl)*; 122(3):223-9.

Snook, J., Boothman, B.D., Watkins, J., *et al.* 1988. Torsade de point ventricular tachycardia associated with astemizole overdose. *Br. J. Clin. Pract.*; 42:257-259.

Sobel, J. D. 1986. Recurrent vulvovaginal candidiasis: a prospective study of the efficacy of maintaince ketoconazole therapy. *N. Engl. J. Med.* 315:1455-1458.

Sproule, B.A., Naranjo, C.A., Brenmer, K.E., *et al.* 1997. Selective serotonin reuptake inhibitors and CNS drug interactions. A critical review of the evidence. *Clin Pharmacokinet.*; 33(6):454-71.

Stresser, D.M., Kupfer, D. 1999. Monospecific antipeptide antibody to cytochrome P-450 2B6. *Drug Metab Dispos.*; 27:517-25.

Suzuki, S., Kurata, N., Nishimura, Y., *et al.* 2000. Effects of imidazole antimycotics on the liver microsomal cytochrome P450 isoforms in rats: comparison of *in vitro* and *ex vivo* studies. *Eur J Drug Metab Pharmacokinet.*; 25(2):121-6.

Swart, P.J., Krauwinkel, W.J., Smulders, R.A., *et al.* 2006. Pharmacokinetic effect of ketoconazole on solifenacin in healthy volunteers. *Basic Clin Pharmacol Toxicol.;* 99(1):33-6.

Tailor, S., Gupta, A., Walker, S., *et al.* 1996. Peripheral edema due to nifedipine-itraconazole interaction : a case report. *Arch. Dermatol.*; 132:350-352.

Takano, M., Hasegawa, R., Fukuda, T., *et al.* 1998. Interaction with P-glycoprotein and transport of erythromycin, midazolam and ketoconazole in Caco-2 cells. *Eur J Pharmacol*; 358, 289–294.

Talbot, M.D., and Spencer, R.C. 1983. Oral ketoconazole in the treatment of vaginal candidiasis. *Cur. Ther. Res.*; 34:746-749.

Tanaka, E. 1998. Clinically important pharmacokinetic drug-drug interactions: role of cytochrome P450 enzymes. *J Clin Pharm Ther.*; 23:403-16.

Theuretzbacher, U., Ihle, F., Derendorf, H. 2006. Pharmacokinetic/ pharmacodynamic profile of voriconazole. *Clin Pharmacokinet.*; 45:649-63.

Thummel, K.E., Shen, D.D., Podoll, T.D. 1994. Use of midazolam as a human cytochrome P450 3A probe: I. *In vitro-in vivo* correlations in liver transplant patients. *J Pharmacol Exp Ther.*; 271: 549-556.

Thummel, K.E., Wilkinson, G.R. 1998. *In vitro* and *in vivo* drug interactions involving human CYP3A. *Annu Rev Pharmacol Toxicol.*; 38:389-430.

Totah, R.A., Rettie, A.E. 2005. Cytochrome P450 2C8: substrates, inhibitors, pharmacogenetics, and clinical relevance. *Clin Pharmacol Ther.*; 77:341-52.

Travers, D., Levoyer, D., Millet, B. 2008. Insight in schizophrenia: assessment of 31 patients with different scales. *Encephale.*; 34(1):66-72.

Tsunoda, S.M., Velez, R.L., von Moltke, L.L., *et al.* 1999. Differentiation of intestinal and hepatic cytochrome P450 3A activity with use of imidazolam as in-vivo probe: effect of ketoconazole. *Clin. Pharmacol. Ther.*; 66:461-471.

Turpeinen, M., Nieminen, R., Juntunen, T., *et al.* 2004. Selective inhibition of CYP2B6-catalyzed bupropion hydroxylation in human liver microsomes in vitro. *Drug Metab Dispos.*; 32:626-31.

Turpeinen, M., Raunio, H., Pelkonen, O. 2006. The functional role of CYP2B6 in human drug metabolism: substrates and inhibitors *in vitro*, *in vivo* and in silico. *Curr Drug Metab.*; 7:705-14.

Turpeinen, M., Tolonen, A., Uusitalo, J., *et al.* 2005. Effect of clopidogrel and ticlopidine on cytochrome P450 2B6 activity as measured by bupropion hydroxylation. *Clin Pharmacol Ther.*; 77:553-9.

Urichuk, L., Prior, T.I., Dursun, S., *et al.* 2008. Metabolism of atypical antipsychotics: involvement of cytochrome p450 enzymes and relevance for drug-drug interactions. *Curr Drug Metab.*; 9(5):410-8.

Van der Meer, J.W.M., Kening, J.J., Scheijrond *et al.*, 1980. Influnce of gastric acidity on the bioavailability of ketoconazole. *J. Antimicrob. Chemother.*; 6:522-524.

Van der Weide, J., Steijns. L.S, van Weelden. M.J., *et al.* 2001. Th e effect of genetic polymorphism of cytochrome P450 CYP2C9 on phenytoin dose requirement. *Pharmacogenetics*; 11:287-91.

Van Tyle, J.H. 1984. Ketoconazole, mechanism of action spectrum of activity pharmacokinetics, drug interaction, adverse reaction and therapeutic use. *Pharmacotherapy;* 4: 343-373.

Varhe, A., Olkkola, K.T., Neuvonen, P.J. 1994. Oral triazolam is potentiatially hazardous to patients receiving systemic antimycotics ketoconazole or itraconazole. *Clin. Pharmacol. Ther.;* 56:601-607.

Venkatakrishnan, K., von Moltke, L.L., Greenblatt, D.J., *et al.* 2000. Effects of the antifungal agents on oxidative drug metabolism: clinical relevance. *Clin Pharmacokinet.;* 38(2 2):111-80.

Venkatakrishnan, K, Von Moltke, L.L, Greenblatt, D.J. 2001. Human drug metabolism and the cytochromes P450: application and relevance of in vitro models. *J Clin Pharmacol.* 41:1149-79.

Vermeir, M., Naessens, I., Remmerie, B., *et al.* 2008. Absorption, metabolism, and excretion of paliperidone, a new monoaminergic antagonist, in humans. *Drug Metab Dispos.;* 36(4):769-79.

Vidal-Puig, A.J., Munoz-Torres, M., Jodar-Gimeno, E., *et al.* 1994. Ketoconazole therapy: hormonal and clinical effect in non-tumoral hyperandrogenism. *Eur. J. Endocrinol.;* 130:333-338.

von Moltke, L.L., Granda, B.W., Grassi, J.M., et al. 2004. Interaction of triazolam and ketoconazole in P-glycoprotein-deficient mice. J. Pharmacol. Exp. Ther.; 32(8):8000-804.

Wacher, V.J., Silverman, J.A., Zhang, Y., *et al.* 1998. Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. *J Pharm Sci.;* 87:1322–1330.

Walsky, R.L., Obach, R.S., Gaman, E.A. 2005b. Selective inhibition of human cytochrome P4502C8 by montelukast. *Drug Metab Dispos.;* 33:413-8.

Wang, J.S., Neuvonen, M., Wen, X., *et al.* 2002. Gemfibrozil inhibits CYP2C8-mediated cerivastatin metabolism in human liver microsomes. *Drug Metab Dispos.;* 30:1352-6.

Wang, Y.H., Jones, D.R., Hall, S.D. 2004. Prediction of cytochrome P450 3A inhibition by verapamil enantiomers and their metabolites. *Drug Metab Dispos.;* **32:**259–266.

Watanabe, M., Kumai, T., Matsumoto, N., *et al.* 2004. Expression of CYP3A4 mRNA is correlated with CYP3A4 protein level and metabolic activity in human liver. J Pharmacol Sci.; 94(4):459-62.

Ward, B.A., Gorski, J.C., Jones, D.R., *et al.* 2003. The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. *J Pharmacol Exp Ther.*; 306:287-300.

Watkins, P.B. 1997. The barrier function of CYP3A4 and P-glycoprotein in the small bowel. *Adv Drug Deliv Rev.*; (27), 161–170.

Wen, X., Wang J.S., Backman, J.T., *et al.* 2002. Trimethoprim and sulfamethoxazole are selective inhibitors of CYP2C8 and CYP2C9, respectively. *Drug Metab Dispos.*; 30:631-5.

Werck-Reichhart, D., Feyereisen, R. 2000. Cytochromes P450: a success story. *Genome Biol.* 1:REVIEWS.3003.

Whitten, J.R., Ruehter, V.L. 1997. Risperidone and hyponatremia: a case report. *Ann Clin Psychiatry*; 9(3):181-3.

Wilkinson, G.R. 2005. Drug metabolism and variability among patients in drug response. *N Engl J Med.*; 352:2211-21.

Williams, R., 2001. Optimal dosing with risperidone: updated recommendations. *J Clin Psychiatry*; 62(4):282-9.

Willson, T.M., Kliewer, S.A. 2002. PXR, CAR and drug metabolism. *Nat Rev Drug Discov.*; 1:259-66.

Wrighton, S.A., Schuetz, E.G., Thummel, K.E., *et al.* 2000. The human CYP3A subfamily: practical considerations. *Drug Metab Rev.*; 32:339-61.

Wrighton, S.A., Stevens, J.C. 1992. The human hepatic cytochromes P450 involved in drug metabolism. *Crit Rev Toxicol.*; 22:1-21.

Wrighton, S.A., Thummel, K.E. 2000. CYP3A. In: Levy RH, Thummel KE, Trager WF, Hansten PD, Eichelbaum M, editors. *Metabolic drug interactions.* Philadelphia: Lippincott Williams & Wilkins. pp. 115-33.

Xu, C., Li, C.Y., Kong, A.N. 2005. Induction of phase I, II and III drug metabolism/transport by xenobiotics. *Arch Pharm Res.*; 28:249-68.

Yoshimura, R., Kakihara, S., Umene-Nakano, W., *et al.* 2008. Acute risperidone treatment did not increase daily cigarette consumption or plasma levels of cotinine and caffeine: a pilot study. *Hum Psychopharmacol.* 2008; 23(4):327-32.

Zhang, Q.Y., Dunbar, D., Ostrowska, A., *et al.* 1999. Characterization of human small intestinal cytochromes P-450. *Drug Metab Dispos.*; 27:804-9.

Zanger, U.M., Eichelbaum, M. 2000. CYP2D6. In: Levy RH, Thummel KE, Trager WF, Hansten PD, Eichelbaum M, editors. *Metabolic drug interactions.*; Philadelphia: Lippincott Williams & Wilkins. pp. 87-94.

Zanger, U.M., Raimundo, S., Eichelbaum, M. 2004. Cytochrome P450 2D6: overview and update on pharmacology, genetics, biochemistry. *Naunyn Schmiedebergs Arch Pharmacol.*; 369:23-37.

APPENDICES

APPENDIX I

Plasma concentrations of risperidone in each of ten subjects receiving a single oral dose of 2 mg (Phase 1).

Time	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Mean
0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.18954	0.32619	2.7	0	0	0	0.95887	0.4	0.0860	0.96404	0.56246
0.5	15.4092	0.97654	8.95	0	6.0413	6.03	9.20251	4.76	0.8460	12.6129	6.48283
0.75	12.6081	7.12762	23.46	0.9309	16.5289	12.63	21.992	7.79	9.0059	22.7351	13.4809
1	19.5537	14.9932	12.12	5.8237	5.4627	13.88	16.5819	7.92	18.7460	23.7332	13.8814
1.5	11.1268	11.4503	8.68	13.6448	8.1214	10.99	13.5157	6.78	16.8454	19.5957	12.075
2	7.17306	9.77583	7.09	9.0587	7.5104	9.3	12.4878	7.42	17.0436	18.8752	10.5735
3	8.31339	10.1837	7.82	7.4771	3.1362	8.73	11.3057	3.48	15.3391	16.278	9.20631
4	5.77552	10.688	4.99	3.5476	2.1372	6.71	12.3639	3.06	12.5390	11.9197	7.37309
5	5.45899	9.33021	4.53	2.7688	3.2992	4.07	8.466	2.52	10.4766	10.0975	6.10173
6	2.76233	7.52923	4.9	2.838	1.7025	3.28	7.42052	1.69	7.3013	8.76797	4.81918
8	1.88867	7.18919	3.44	0.5996	0.2075	1.88	5.37757	1.22	4.8342	6.42152	3.30583
12	0.89272	4.73198	0.33	0	0	0.3	3.06711	0.28	1.3531	2.48696	1.34418
24	0.11308	2.15464	0	0	0	0	0.6017	0	0.3741	0.58141	0.38249
48	0	0.63852	0	0	0	0	0	0	0	0.18449	0.0823
72	0	0.5142	0	0	0	0	0	0	0	0	0.05142
96	0	0	0	0	0	0	0	0	0	0	0

127

Plasma concentrations of risperidone in each of ten subjects receiving a single oral dose of 2 mg after pretreatment with ketoconazole 200 mg for 3 days (Phase 2).

Time	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Mean
0	0	0	0	0	0	0	0	0	0	0	0
0.25	0.53633	0.2418	0.9148	0	0	0	0.2618	0	1.4099	4.5250	0.78897
0.5	9.3935	1.8586	15.3242	0.52	4.27	8.9936	11.4885	5.4333	9.34847	19.6224	8.62526
0.75	14.0862	3.4426	10.8229	13.11	8.27	16.8642	23.4837	14.2807	14.5638	29.1571	14.8081
1	16.6434	6.5591	12.5984	13.75	7.51	23.8779	22.2460	18.542	14.4002	25.7124	16.1839
1.5	15.1291	23.0288	5.7841	10.47	7.6	21.1872	17.7896	15.3518	13.5078	23.7092	15.3558
2	11.8975	11.3002	2.9996	9.68	4.4	19.5976	15.6147	11.0877	10.0493	24.0485	12.0675
3	10.3383	14.9221	4.3771	9.83	2.62	14.285	13.0539	10.3954	12.4288	19.2544	11.1505
4	8.58143	10.8289	0.5142	8.42	3.18	9.8467	10.6319	5.9264	13.4666	18.6242	9.00203
5	6.68833	6.6030	0.5142	4.68	1.55	5.7544	11.5999	6.1926	9.18475	18.0892	7.08563
6	6.06588	8.6763	0.7152	4.4	1.41	7.2316	12.1178	5.0102	6.76814	12.8104	6.52055
8	4.91417	4.4193	0	3.85	0.59	2.5769	8.5659	1.8189	7.08298	10.1647	4.39829
12	2.03785	2.3146	0	0.53	0.1	0.3544	2.9190	0.4933	2.7491	4.3588	1.58571
24	0.56848	0.4820	0	0	0	0	0.5771	0	1.60491	0.8478	0.40803
48	0	0.0777	0	0	0	0	0	0	0.52655	0.2618	0.08661
72	0	0.0496	0	0	0	0	0	0	0	0.1370	0.01867
96	0	0.0571	0	0	0	0	0	0	0	0	0.00571

APPENDIX III

Plasma concentrations of 9-hydroxyrisperidone in each of ten subjects receiving a single oral dose of 2 mg (Phase 1).

Time	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Mean
0	0	0	0	0	0	0	0.0000	0	0.0000	0.0000	0
0.25	0	0	0	0	0	0	0.0000	0	0.0000	0.0000	0
0.5	1.545231	0	5.29	0.02	4.47	5.0353	1.9524	1.6184	0.1889	2.4498	2.257003
0.75	5.045075	0.326264	13.13	7.85	12.77	11.783	7.2760	7.4485	2.2904	5.4051	7.332411
1	8.594993	0.626048	15	11.5	14.25	21.425	7.4475	12.876	8.2806	4.8720	10.48721
1.5	10.11842	0.505942	13.18	9.22	18.61	28.701	10.1117	14.133	11.4779	6.4591	12.25168
2	7.710959	2.493469	12.35	9.72	17.1	27.041	8.5236	12.294	13.5599	7.9950	11.87879
3	9.840139	1.394541	14.85	14.41	13.09	28.354	10.0568	15.013	15.3819	8.3037	13.06939
4	9.227314	1.657782	16.84	14.5	15.65	27.12	11.0318	11.862	15.6909	8.6863	13.22665
5	9.76755	2.625625	16.04	15.16	15.08	26.676	14.7863	14.801	18.9807	12.0612	14.59781
6	10.91017	2.196504	12.64	18.96	15.3	27.863	17.9142	15.868	13.4729	11.8261	14.69512
8	10.95492	2.937826	12.64	20.58	12.34	26.9	15.6856	12.232	13.2636	15.4300	14.29634
12	8.028298	4.493467	9.26	13.38	9.29	22.59	13.1227	10.652	11.6538	11.9204	11.43905
24	7.081181	1.920859	5.09	7.18	3.96	12.001	9.1207	5.0825	8.6483	6.8400	6.692491
48	4.144836	0.844162	1.54	1.79	1.29	3.2177	2.9052	2.0274	3.6178	2.4230	2.379999
72	1.277217	0.990584	0.75	0.63	0	1.424	0.9168	0.5952	2.8699	0.8556	1.030928
96	0.862409	0.260557	0.31	0.25	0	0.2376	0.4587	0.0025	0.6716	0.4965	0.35499

Plasma concentrations of 9-hydroxyrisperidone in each of ten subjects receiving a single oral dose of 2 mg after pretreatment with ketoconazole 200 mg for 3 days (Phase 2).

Time	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Mean
0	0	0.0000	0	0	0	0	0	0	0	0	0
0.25	0.02757	0.0000	0.4699	0	0	0	0	0.12	0	0	0.061747
0.5	4.694024	0.5790	6.6693	0	4.6045	4.04	1.2562	2.72	0.993519	0.940	2.649656
0.75	5.23686	1.1359	8.5437	0.3299	23.781	11.62	7.992165	5.57	2.265516	2.405	6.887984
1	10.35301	1.9323	23.927	1.9104	14.999	15.17	5.866935	6.77	3.067516	1.735	8.57309
1.5	5.111974	12.1552	9.7466	11.984	16.666	16.86	6.407668	5.89	4.015163	2.639	9.147507
2	2.035157	3.7991	4.2882	8.1145	18.864	18.76	7.040352	9.05	3.007567	2.968	7.792738
3	4.535901	6.1696	3.1825	12.302	14.983	18.16	5.8812	5.67	3.68803	3.116	7.768781
4	2.782021	5.5763	2.5125	8.9495	14.253	19.43	8.956899	7.72	3.148422	0.940	7.426856
5	2.497417	1.8825	2.5125	12.1	10.928	16.99	7.853307	6.66	4.719309	1.380	6.752327
6	3.190869	4.9287	8.435	15.855	21.378	14.78	6.642979	6.54	4.492013	3.066	8.930834
8	2.176497	2.6864	8.0618	8.3345	13.302	15.79	9.584336	7.3	3.772792	3.172	7.418015
12	2.168905	3.9264	9.6329	4.7487	6.2003	11.87	10.34534	4.95	6.089594	2.097	6.202926
24	1.906397	0.8444	2.6274	2.4932	1.4909	8.45	3.50415	3.53	4.193774	1.979	3.101941
48	0.337565	0.8310	0.4243	0.7865	0.7865	3.23	1.42156	0.69	1.008926	0.434	0.995034
72	0.167024	0.4021	0.0683	0	0.023	1	1.161287	0.25	0.326963	0	0.339865
96	0.050438	0.1214	0.03	0	0	0.63	0.478325	0.13	0.238776	0	0.167892

APPENDIX V

หนังสือรับรองโครงการวิจัยจากคณะกรรมการจริยธรรมการวิจัยที่ทดลองในมนุษย์

วท-จธ/50/9-1

ที่ ศธ 0521.1.09/ 090

คณะวิทยาศาสตร์
มหาวิทยาลัยสงขลานครินทร์
ตู้ ปณ 3 คอหงส์ 90112

หนังสือรับรองโครงการวิจัย

การศึกษาวิจัยที่ทำการทดลองในมนุษย์เรื่อง : " ผลของคีโตโคนาโซลต่อเภสัชจลนศาสตร์ของริสเพอริโดนใน
อาสาสมัครชายไทยสุขภาพปกติ "

หัวหน้าโครงการวิจัย : ผศ.นายแพทย์วีรวัฒน์ มหัทธนตระกูล
ภาควิชาเภสัชวิทยา คณะวิทยาศาสตร์

ได้ผ่านการพิจารณาและเห็นชอบจาก คณะกรรมการจริยธรรมการวิจัยที่ทดลองในมนุษย์ คณะวิทยาศาสตร์

ให้ไว้ ณ วันที่ 8 กุมภาพันธ์ 2550

(รองศาสตราจารย์ ดร.วิไลวรรณ โชติเกียรติ)
รองคณบดีฝ่ายวิจัยและบัณฑิตศึกษา ปฏิบัติราชการแทน
คณบดีคณะวิทยาศาสตร์

131

ACKNOWLEDGEMENT