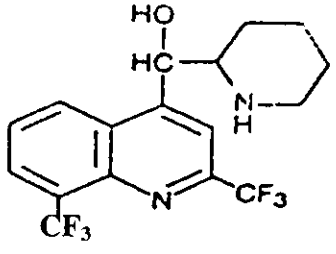
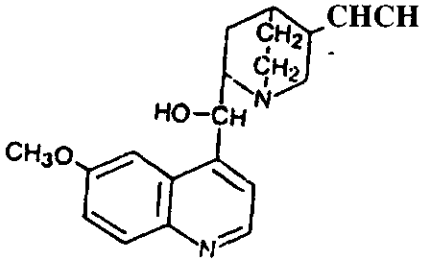
# LITERATURE REVIEW

## Mefloquine

Mefloquine[*dl*-erythro-a-(2-piperidyl) -2 ,8 -bis ( trifluoromethyl) -4 -quinoline methanol] is a quinoline methanol which structurally related to quinine (Figure 1) and selected for development by the Walter Reed Army Institute of Research in the US from over 30,000 screened compounds. Although it was only marketed in 1985, it is probably the most frequently studied of all antimalarials (Nosten, *et al.*, 1995).




Mefloquine




Quinine

**Figure 1** Structural formula of mefloquine and quinine

Mefloquine has a molecular weight of 414.79 and is a very lipid-soluble. It is insoluble in water. Mefloquine is a basic, highly lipophilic drug (partition coefficient n-octanol/buffer pH 7.4= 630), with low aqueous solubility at

physiological pH (60 mg/100 ml at pH 1.2 and 30 mg/100 ml at pH 7.4) (Crevoisier, et al., 1997).

## 1. Pharmacodynamic properties

### 1.1 Mechanism of Action

The mechanism of action of mefloquine is unknown. In many respects, mefloquine behaves like quinine, but it does not intercalate with DNA. Mefloquine and quinine produce similar morphological changes in early ring stages of *Plasmodium falciparum* and *Plasmodium vivax,* the major ultrastructural abnormality produced by mefloquine in *P. falciparum* is swelling of secondary lysosomes. Like chloroquine, low extracellular concentrations of mefloquine raise the intravesicular pH of plasmodia in excess of that predicted from the passive distribution of a weak base. This suggests that there is a mechanism for concentration of mefloquine that has to be characterized. Mefloquine probably affects membranes of malarial parasites by both inhibiting heme polymerization and forming toxic complexes with free heme that damage membranes and interact with other plasmodial components. The orientation of the hydroxyl and amine groups with respect to each other in mefloquine may be essential for its hydrogen bonding and antimalarial activity Goldsmith, 2000). Like quinine, mefloquine competes for accumulation of chloroquine in infected erythrocytes and inhibits chloroquine-induced clumping of pigment in the parasite (Webstar, 1991).

### 1.2 Pharmacologic Effects

Like quinine, mefloquine can slow cardiac conduction. Studies in animals have shown antifibrillary action and an increase in the pulse rate interval. The effect of mefloquine on the compromised cardiovascular system in humans has

not been evaluated (Goldsmith, 1992). Mefloquine was found to disrupt neuronal calcium homeostasis and in due an ER stress response at physiologically relevant concentrations, effect that may contribute, at least in part, to the neurotoxicity of the drug. At physiologically relevant concentrations mefloquine was found to mobilize neuronal ER calcium stores and antagonize the pharmacological action of thapsigargin, a specific inhibitor of the ER calcium pump. Mefloquine also induced a sustained influx of extra-neuronal calcium via an unknown mechanism. These effects appear to be related, in terms of dose effect and kinetics of action, to the acute neurotoxicity of the drug *in vitro* (Dow, et al., 2003)

1.3 Antimalarial Action

Mefloquine has strong blood schizonticidal activity against *P.falciparum* and *P. vivax.* Insufficient information is available to document effectiveness against *P. malariae* or *P. ovale,* but theoretically the drug should be effective against circulating schizonts of these species (Goldsmith, 2000).

1.4 Resistance

Sporadic and low levels of resistance to mefloquine have been reported from Southeast Asia and Africa. Resistance to the drug can emerge rapidly, and resistant strains have been found in areas where the drug has never been used (Goldsmith, 2000).

The first case of confirmed prophylaxis failure due to mefloquine resistance in East Africa. The reporting the case of a 44-year-old German female traveled to Tanzania for 3 weeks. The patient reported that she never missed a dose of mefloquine during her weekly prophylaxis schedule. The patient presented witrh fever, headache and myalgia. Only a few trophozoites of *Plasmodium faciparum*

were found in thick film. Blood level of mefloquine at that stage were at 1400 ng/ml, thus largely excluding non-compliance and malabsorption (Wichmann et al., 2002)

Cross-resistance between mefloquine and halofantrine and cross-resistance between mefloquine and quinine has been observed in some regions (Hoffmann. 2002).

## 2. Pharmacokinetic properties

Mefloquine hydrochloride is a synthetic 4-quinoline methanol derivative chemically related to quinine. It can only be given orally because intense local irritation occurs with parenteral use. It is well absorbed, and peak plasma concentrations are reached in 7-24 hours (Goldsmith, 2000). The bioavailability of commercial preparations exceeds 85%. Absorption is enhanced by food and impaired by vomiting within the first hour of ingestion and diarrhea in the following days (Nosten, et al., 1995). A single oral dose of 250 mg of the salt results in a plasma concentration of 290-340 ng/ml whereas continuation of this dose daily results in mean steady state plasma concentrations of 560-1,250 ng/ml. 1 Plasma levels of 200-300 ng/ml may be necessary to achieve chemosuppression in *P. falciparum* infections (Goldsmith, 2000). The drug is highly bound to plasma proteins (98%) (Palmer, et al., 1993; Webstar, 1991; Hoffmann, 2002). Estimate of apparent volume of distribution is 20 L/Kg (Palmer, et al., 1993; Hoffmann, 2002). The drug concentrated in red blood cells, and extensively distributed to the tissues, including the central nervous system (Goldsmith, 2000). Mefloquine is cleared in the liver, it under goes extensively to biliary and gastric secretion followed by reabsorption (Strickland, et al., 1991). Its acid metabolites are slowly excreted, mainly in the feces and only very small amounts of drug appear in the

urine. Its elimination half-life, which varies from 13 days to 33 days, tends to be shortened in patients with acute malaria. The drug can be detected in the blood for months after dosing ceases. These findings are consistent with the large volume of distribution and low clearance drug (Goldsmith, 2000; Webstar, 1991). In rats, the gastrointestinal system serves as an important compartment for the drug as it undergoes a continuous enterohepatic and enterogastric circulation (Webstar, 1991). Clearance is increased in young children and late pregnancy (Nosten, et al., 1995). Pharmacokinetic parameters between Asian and Caucasian are different. Although the reason for this ethnic variation has not been investigated, it has been suggested that a smaller volume of distribution, secondary to a relatively lower body fat content, or differences in the enterohepatic circulation of mefloquine could be accounted for higher blood drug concentrations observed in Asians (Palmer, et al., 1993).

## 2.1 Studies in Healthy Volunteers

### 2.1.1 Absorption

The absolute oral bioavailability of mefloquine has not been determined since an intravenous formulation is not available. However, the bioavailability of an early tablet formulation was determined from a comparison of the respective area under the plasma concentration-time curve (AUC) values of the tablet formulation relative to an aqueous suspension of mefloquine hydrochloride, and was estimated to be 65% in healthy Caucasian volunteers. A subsequent tablet formulation showed improved bioavailability, estimated at 89% in healthy Caucasian volunteers and 87% in Thai patients with malaria using a stable isotope technique (Palmer, et al., 1993).

The presence of food affects the pharmacokinetic properties of mefloquine, significantly enhancing the rate and extent of absorption, as evidenced by an increase in $C_{max}$ and reduction in time taken to reach $C_{max}$ ($t_{max}$) and a 40% increase in bioavailability (Crevoisier, et al., 1997). These data suggest that mefloquine should be taken at a standard time in relation to meals to minimize variation in blood concentrations (Palmer, et al., 1993).

### 2.1.2 Distribution

In healthy adults, the apparent volume of distribution is approximately 20 L/kg, indicating extensive tissue distribution. Mefloquine may accumulate in parasitized erythrocytes. Experiments conducted in vitro with human blood using concentrations between 50 and 1000 mg/mL showed a relatively constant erythrocyte-to-plasma concentration ratio of about 2 to 1. The equilibrium reached in less than 30 minutes, was found to be reversible. The plasma proteins in volunteers (98.2%) and patients (98.4%) (Palmer, et al., 1993; Hoffmann, 2002)

### 2.1.3 Elimination

In several studies in healthy adults, the mean elimination half-life of mefloquine varied between 2 and 4 weeks, with an average of about 3 weeks. Total clearance, which is essentially hepatic, is in the order of 30 mL/min. There is evidence that mefloquine is excreted mainly in the bile and feces. In volunteers, urinary excretion of unchanged mefloquine and its main metabolite under steady-state condition accounted for about 9% and 4% of the dose, respectively. Concentrations of other metabolites could not be measured in the urine.

## 2.2 Studies in Patients with Malaria

### 2.2.1 Absorption

Malaria does not appear to impair the absorption of mefloquine, indeed plasma $C_{max}$ were higher in patients with acute uncomplicated falciparum malaria compared with uninfected individuals. Although ethnic differences may have contributed to the higher plasma $C_{max}$, recent studies directly comparing mefloquine kinetics in infected and uninfected Thai patients have demonstrated similar or higher plasma $C_{max}$ values in patients with acute uncomplicated falciparum malaria (Boudreau, et al., 1990; Palmer, et al., 1993).

### 2.2.2 Distribution and Elimination

A comparison of overall means derived from pharmacokinetic investigations in patients with falciparum malaria and healthy volunteers suggests that the terminal elimination half-life and apparent volume of distribution of mefloquine are reduced in malarial infection (Palmer, et al., 1993). Karbwang, et al. (1988) proposed that enterohepatic recirculation might be augmented during malarial infection. Palmer, et al., (1993) suggested that the fever and parasitaemia associated with severe infection affected the distribution of mefloquine. Indeed, malarial parasites are known to concentrate antimalarial drugs and this accumulation probably contributes to the low apparent volume of distribution during the first 3 days of treatment (Palmer, et al., 1993)

## 2.3 Studies in Children and Pregnant Woman

A more recent study demonstrated that following mefloquine 25 nig/kg, $C_{max}$ , $t_{max}$, elimination half-life, volume of distribution and AUC in children aged 6 to 24 months were similar to those values observed in children aged 5 to 12 years. These values are similar to those in adults with falciparum malaria treated

with a comparable dose (750 mg) suggesting that there are no age-related differences in mefloquine disposition (Palmer, et al., 1993; Dubos, et al., 2003).

Pharmacokinetic studies of mefloquine have been performed in pregnant woman, assuming bioavailability was not reduced; systemic clearance was increased in late pregnancy compared with that observed in healthy no pregnant adults. The quantity of mefloquine excreted in human breast milk after a maternal dose of 250 mg is unlikely to be harmful to a breasted infant (Palmer, et al., 1993)

2.4 Studies in patients with end-stage renal disease

The two patients, short-term travelers to area where malaria is endemic, took 250 mg of mefloquine (Larium) once weekly for 2 weeks before and during their 3 week stay aboard and for one week after their return. Pre- and postdialysis blood sample were drawn before their departure and after their return. The concentration time profile of mefloquine and carboxylic acid metabolite could not be detected in the dialysate. These finding show that mefloquine and carboxylic acid metabolite are not, or are very poorly, removed by hemodialysis. Concentrations in plasma and accumulation kinetics were similar to those reported for healthy volunteers and were associated with high prophylactic efficacy against malaria (Crevoisier, et al., 1995).

2.5 Metabolism

Data on the metabolism of mefloquine in humans are limited. Animal studies indicated that mefloquine is metabolised in the liver, being excreted predominantly in the bile and faeces. In human volunteers the plasma concentration of a primary human mefloquine metabolite, the 4-carboxylic acid derivative (Ro21-5104), exceeds that of the parent compound after single dose, divided dose and multiple dose administration (Karbwang and White, 1990;

Palmer, et al., 1993). In rodents, parenterally administered [$^{14}$C]-lapelled drug was excreted primarily in the faeces; 30% appeared as unchanged drug in the mouse, but virtually no parent drug was excreted in the rat. In these studies, mefloquine was well absorbed, extensively distributed throughout the body, and concentrated in the bile and gastric juice. Five metabolites have been isolated from the faeces of rats, two of which have been identified as 2,8 bis-trifluoromethyl 4-hydroxymethyl quinoline and a carboxylic metabolite. This suggests that biotransformation occurs in the piperidine ring but not in the aromatic portion of the mefloquine molecule. The structurally defined components account for about 40% of the administered dose.

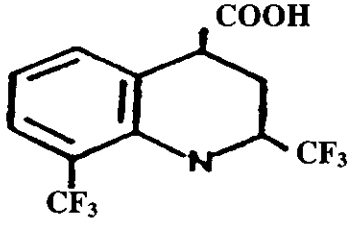



**Figure 2** Structural formula of a carboxylic metabolite

These workers concluded that the relatively high plasma concentrations of the mefloquine metabolite observed after oral administration in humans are explained by its smaller apparent volume of distribution (Vd/F) (Karbwang and White, 1990)

Mefloquine is largely biotransformed into 2,8-bis (trifluoromethyl)-4-quinoline carboxylic acid (Figure 2). Schwartz, et al. (1980) studies the disposition of the carboxylic acid metabolite in human subjects given 1 g mefloquine base in the form of its hydrochloride orally. The metabolite appeared

in the blood 2 to 4 hours after oral administration of mefloquine. Its concentration rose steadily to maximum values of 1.1 to 1.4 μg/ml within 1 or 2 weeks of administration and remained practically constant for about 2 to 3 weeks to decline thereafter at a rate similar to that of mefloquine. The authors compared the kinetic studies of 250 mg mefloquine and 204 mg of its metabolite in dogs, using i.v. administration. In these experiments, both compounds showed virtually the same elimination half-life but the initial plasma levels of mefloquine metabolite was about 30 times higher than those of mefloquine. The volume of distribution of this compound was less than that of mefloquine. On comparing the AUC of metabolite formed after i.v. administration of 250 mg mefloquine with that measured after i.v. administration of 204 mg of metabolite, it was found that only 26% of mefloquine had been metabolically converted to mefloquine metabolite. These workers concluded that the relatively high plasma concentrations of the mefloquine metabolite observed after oral administration in humans are explained by its smaller apparent volume of distribution (Vd/F) (Karbwang and White, 1990). Schwartz, et al. (1982) studied the oral single dose kinetics of mefloquine in 16 male volunteers. Unchanged mefloquine and one of its metabolites were measured in plasma. The levels of its metabolite surpassed those of mefloquine, resulting in a 2.4-5.1 larger AUC. Franssen, et al. (1989) reported that the concentration of the metabolite in whole blood was lower than mat in plasma but also exceeded the whole blood concentration of mefloquine. Mimica, et al. (1983) reported steady-state plasma concentration of mefloquine and carboxylic acid metabolite in 5 volunteers receiving 250 mg weekly for 21 weeks. The mean plasma levels of the metabolite ranged between 1.47 and 5.55 μg/ml, while the

mean metabolite to mefloquine ratio measured at steady state was found to have an inter individual range of between 2.3 and 8.6.

3. Toxicology

UNDP/World Bank/WHO Updated 983) reported the toxicity of mefloquine in the rats, dogs, monkeys and mice. The results of acute, sub acute and chronic toxicity showed that the drug did not cause mutagenic nor teratologic changes. The toxic effects on the development of the offspring of rats were produced during the postnatal period only in those nursed by dams given very high doses of the drug. There was no evidence of toxic effects in dogs given doses ranging from 5 to 150 mg/kg/week for 1 year, other than a reduction in the rate of weight gain in those receiving 25 mg/kg or more of drug per week. When the drug was given for 28 consecutive days, no adverse effects were noted at doses of 5 mg/kg/day, and the only abnormality detected at doses of 30 mg/kg/day was slight lymphocytopenia. However, doses of 150 mg/kg/day were lethal within 2 weeks. In male rats daily doses of more than 20 mg/kg for 13 weeks induced degenerative lesions in the epididymis. Teratological studies in female rats showed an increased incidence of externally visible soft tissue and skeletal anomalies when the drug was given orally in very large doses (100 mg/kg) from day 6 to day 15 of gestation, but no abnormalities were seen at doses of 10 or 20 mg/kg. Similar doses in mice reduced fetal growth and produced cleft palate in some offspring, a 2-year study in rats showed that mefloquine is not carcinogenic at doses of 30 mg/kg/day or below. Thus, it possesses a relatively satisfactory overall profile of animal toxicity (Karbwang and White, 1990).

4. Adverse Reactions

Mefloquine, given orally in single doses up to 1,500 mg or in 500 mg doses each week for 1 year, it generally well tolerated (Webstar, 1991). The frequency and intensity of reactions are dose-related. Most of the symptoms, however, can also occur as a result of malaria itself.

4.1 Prophylactic Doses: Minor and transient adverse effects include gastrointestinal disturbances (nausea, vomiting, epigastric pain, diarrhea), headache, dizziness, syncope and extrasystoles, with an incidence not much higher than for placebo or other antimalarials. Transient leukocytosis, thrombocytopenia, and aminotransferase elevations have been reported. Transient neuropsychiatric events (convulsions, depression, psychoses) occur about 1 in 13,000 patients taking it for prophylaxis (Ottawa. 2002).

4.2 Treatment Doses: Particularly with therapeutic doses over 1,000 mg, gastrointestinal symptoms and fatigue are more likely and the incidence of neuropsychiatric symptoms (dizziness, headache, visual disturbances, tinnitus, insomnia, restlessness, anxiety, depression, confusion, acute psychosis, or seizure) occur in about 4 to 7 patients in 1000 given mefloquine to treat malaria (Ottawa. 2002).

4.3 Animal Toxicity: Ocular lesions (lens opacity, retinal degeneration) have been reported in animals but not in humans.

4.4 Carcinogenesis

The carcinogenic potential of mefloquine was studied in rats and mice in 2-years feeding studies at doses of up to 30 mg/kg/day. No treatment-related increases in tumors of any type were noted.

### 4.5 Mutagenicity

The mutagenic potential of mefloquine was studied in a variety of assay systems including: Ames test, a host-mediated assay in mice, fluctuation tests and a mouse micronucleus assay. Several of these assays were performed with and without prior metabolic activation. In no instance was evidence obtained for the mutagenicity of mefloquine.

### 4.6 Impairment of Fertility

Fertility studies in rats at doses of 5, 20, and 50 mg/kg/day of mefloquine havedemonstrated adverse effects on fertility in the male at the high dose of 50 mg/kg/day, and in the female at doses of 20 and 50 mg/kg/day. Histopathological lesions were noted in the epididymides from male rats at doses of 20 and 50 mg/kg/day. Administration of 250 mg/week of mefloquine (base) in adult males for 22 weeks failed to reveal any deleterious effects on human spermatozoa.

### 4.7 Teratogenicity

Mefloquine has been demonstrated to be teratogenic in rats and mice at a dose of 100 mg/kg/day. In rabbits, a high dose of 160 mg/kg/day was embryotoxic and teratogenic, and a dose of 80 mg/kg/day was teratogenic but not embryotoxic. There are no adequate and well-controlled studies in pregnant women. However, clinical experience with Lariam has not revealed an embryotoxic or teratogenic effect. Mefloquine should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Women of childbearing potential who are traveling to areas where malaria is endemic should be warned against becoming pregnant.

5. Contraindication & Cautions

Mefloquine is contraindicated if there is a history of epilepsy, psychiatric disorders, arrhythmia, cardiac conduction defect, or sensitivity to quinidine or related drugs. The drug should not be used for children under 15 kg or under 2 years of age. In these groups, the drug appears to be poorly tolerated, and efficacy has not been established. Also contraindicated is concurrent administration of mefloquine with quinine, quinidine, or halofantrine. If these agents precede the use of mefloquine, 12 hours should elapse before mefloquine is started. Because of the long half-life of mefloquine, extreme caution is required if quinine or quinidine is used to treat malaria after mefloquine has been taken.

The drug should preferably not be used in the first trimester of pregnancy, and woman of childbearing potential who takes mefloquine for malarial prophylaxis should use reliable precautions against conception for 3 months after the last dose.

The development of neuropsychiatric symptoms (anxiety, depression, restlessness, confusion) during prophylaxis is an indication to stop using mefloquine. Persons should not take the drug whose work requires fine coordination and spatial discrimination (e.g., airline pilots). Patients taking anticonvulsant drugs (particularly valproic acid and divalproex sodium) may have break through seizures; concurrent administration of mefloquine and chloroquine or quinine increases the risk of convulsions.

The safety of mefloquine usage beyond 1 year has not been established. Therefore, if the drug is used for prolonged periods, periodic evaluations,

including liver function tests and complete ophthalmologic examinations, are recommended (Goldsmith, 2000).

6. Clinical Uses

6.1 Prophylaxis of Chloroquine-Resistant Strains of *P. falciparum :*

Dose: One 250 mg tablet salt (228 mg base). Give a single dose of mefloquine weekly starting 1 week before entering the endemic area, while there and for 4 weeks after leaving.

Mefloquine is effective in prophylaxis against most strains of chloroquine-resistant or pyrimethamine- sulfadoxine-resistant *P. falciparum* and is curative when taken weekly for 4 weeks after leaving an endemic area. When used for this purpose, the drug also provides prophylaxis against *P. vivax* and probably against *P. ovale* and *P. malariae.* Eradication of P. *vivax* and *P. ovale,* however, requires a course of primaquine against their hepatic stages (as used with chloroquine in eradicating *P. vivax* infections).

Although the drug is effective against chloroqume-sensitive *P. falciparum,* it should be reserved for use in malarial areas in which chloroquine is not effective.

6.2 Treatment of Chloroquine-Resistant *P.falciparum* Infection :

Dose: Mefloquine, 1,250 mg (salt) once or 750 mg followed after 6-8 hours by 500 mg. Mefloquine is indicated in oral treatment of mild to moderate mefloquine-susceptible *P. falciparum* infections. Since mefloquine apparently does not act as quickly as quinine or quinidine and cannot be given parenterally, it should not supplant these drugs in management of severely ill patients (Goldsmith, 2000)

## 7. Drug Interactions

### Sulfadoxine-Pyrunethamine

Mefloquine is marketed in Thailand in combination with both sulfadoxine and pyrimethamine (Fansimef®). In a recent study comparing the effects of mefloquine alone with those of mefloquine plus sulfadoxine-pyrimethamine, both regimens are equally effective in producing cure (Pinichpongse, *et al.*, 1987). There was significant difference in the half-life for patients who received mefloquine alone and for those receiving mefloquine-sulfadoxine-pyrimethamine. The devived mean half-life of mefloquine was longer following ingestion of "Fansimef" compared to that following mefloquine alone. This suggested that sulfadoxine and/or pyrimethamine may influence mefloquine elimination (Karbwang and White, 1990).

### Quinine

Quinine should not be used concurrently with mefloquine. It can potentiate the dose-related adverse effects of mefloquine. If this compound is to be used in the initial treatment of severe malaria, mefloquine should not be administered within 12 hours of the last dose of quinine (Stem (letter), 1988).

Chantavanich, et al. (1985) showed that mefloquine concentrations rose abruptly when quinine administration ceased. They suggested that competition for plasma and red cell binding sites might explain this finding. The combined effects of quinine and mefloquine on the cardiovascular system warrant urgent investigation so that appropriate treatment recommendation can be made.

### Primaquine

A study was conducted to examine the pharmacokinetics of mefloquine in healthy Thai men who received either mefloquine alone or mefloquine and primaquine. The results suggested that primaquine did not alter the pharmacokinetics of mefloquine in Thai volunteers (Karbwang, et al. (letter), 1992).

### Metoclopamide

A study of the pharmacokinetics of 750 mg mefloquine when administered concomitantly with metoclopamide showed that metoclopamide increased maximum concentration of mefloquine and AUC. This suggested that metoclopamide increased gastrointestinal motility. Since accelerated gastric emptying can lead to an increase in the rate of drug absorption from the small intestine (Na Bangchang, et al., 1991).

### Oral Contraceptive Steroids

The effects of oral contraceptive steroids on the pharmacokinetics of a single oral dose mefloquine (750 mg) were studied in six healthy Thai women volunteers who regularly used oral contraceptive steroids (OCS) and in twelve Thai woman patients with falciparum malaria, six of who were also using OCS. The pharmacokinetic parameters of mefloquine were not significantly different in the two patient groups. The study suggested that the oral contraceptive steroids had no effect on the pharmacokinetics of mefloquine (Karbwang, et al., 1988).

### Ampicillin

Karbwang, et al. (1991) examined the pharmacokinetics of a single oral dose of mefloquine in healthy Thai volunteers who took either mefloquine alone or

in combination with ampicilln. The results showed a significantly higher maximum whole blood mefloquine concentration after co-administration with ampicillin, significantly reduced terminal half-life and increased in mean residence time and increased volume of distribution at steady state. The rationale for this study was to examine the possible role of enterohepatic recycling (EHC) in the prolonged half-life of mefloquine. Although in the ampicillin phase a small decrease in half-life was seen, this was secondary to a decrease in the apparent volume of distribution and the clearance was unchanged. The most likely explanation of this finding is altered tissue binding: although it should be noted that with an intact EHC the gastrointestinal tract will be part of the volume of distribotion, but if EHC is interrupted this will no longer be so.

Cimetidine

Sunbhanich, et al. (1997) investigated the inhibition of the metabolism of mefloquine by cimetidine. Pretreatment with cimetidine 400 mg twice a day after breakfast and at bedtime for 7 days caused markedly significant reduction ($P<0.05$) in the apparent oral clearance (Clo) ($0.051 \pm 0.026$ versus $0.031 \pm 0.015$ 1/h/kg) and a significant increase ($P<0.05$) in the elimination half-life ($t_{1/2}$) after cimetidine treatment (9.6 versus 14.4 days, respectively). Therefore, the present data suggest that cimetidine reduces the Clo and prolongs the elimination $t_{1/2}$ of mefloquine in a manner similar to quinine. These results may reflect the decrease in the metabolism of mefloquine by cimetidine. These effects of cimetidine on the mefloquine pharmacokinetics may be mediated by inhibiting the hepatic-mixed function oxidase system, being similar to those occurring in quinine.

### Valproic acid

When used prophylactically at a dosage of 250 mg mefloquine has been reported to reduce the half-life of valproic acid, possibly by increasing metabolism, resulting in a decrease in seizure control in several epileptic patients. Therefore, concomitant use of these 2 agents is not advised (Palmer, *et al.*, 1993).

### Artemisinin

Studies have shown no clinically significant pharmacokinetic or toxic interactions between the artemisinin compounds and mefloquine when therapy with the latter is begun about 36 to 48 hours after the former. The 1250 mg adult dose of mefloquine is usually split; namely, 750 mg is given after food, followed by 500 mg 12 hours later. A similar strategy with 25 mg/kg of mefloquine, given either as single or split dose on the second day of antimalarial therapy with artesunate, has produced excellent results in children (Price, et al., 1999).

### Rifampin

Ridtitid, et al. (2000) investigated the induction of the metabolism of mefloquine by rifampin. Pretreatment with rifampin 600 mg once daily before breakfast for 7 days (Days 1 – 7), then 600 mg rifampin twice weekly on Days 8-56. The result indicates that rifampin significant decreased the area under the concentrations time curve ($AUC_{0-\square}$) of mefloquine by 68% ($P<0.01$), maximum plasma concentration ($C_{max}$) by 19 % ($P<0.01$) and elimination half life ($t_{1/2}$) by 63% ($P<0.01$), whereas the time to reach $C_{max}$ ($t_{max}$) of mefloquine was unaffected. The apparent oral clearance (CL) of mefliquine was significantly increased by 281% ($P<0.01$). After administration of rifampin, the $C_{max}$ of carboxylic metabolite of mefloquine was significantly increased by 47% ($P<0.05$),

whereas the $t_{1/2}$ was significantly decreased by 39% ($P<0.01$), $t_{max}$ by 76% ($P<0.01$). These results indicate that rifampin reduces the plasma concentration of a single dose of 500 mg mefloquine by increasing metabolism of mefloquine in liver and gut wall.

Human Cardiac Potassium Channel KvLQT1/minK and HERG

Kang, et al. (2001) examined the effects of mefloquine on the slow delayed rectifier $K^+$ channel (KvLQT1/mink) and HERG, the $K^+$ channel that underline the slow ($I_{Ks}$) and rapid ($I_{Kr}$) components of repolarization in the human myocardium, respectively. Mefloquine inhibited KvLQT1/minK channel current with an $IC_{50}$ value of approximately 1 μM. Mefloquine slowed the activation rate of KvLQT1/minK and more blocks were evident at lower membrane potentials compared with higher ones. When channels were held in the closed state during drug application, block was immediate and complete with the first depolarizing step. HERG channel currents were about 6-fold less sensitive to block by mefloquine ($IC_{50}$=5.6 μM). Block of HERG displayed a positive voltage dependence with maximal inhibition obtained at more depolarized potentials. In contrast to structurally related drugs such as quinidine, mefloquine is a more effective antagonist of KvLQT1/mink compared with HERG. Inhibition by mefloquine of KvLQT1/mink in the human heart may in part explain the synergistic prolongation of QT interval observed when this drug is co-administered with the HERG antagonist halofantrine.

Ritonavir

Two studies were performed for the co-administration of ritonavir (200 mg) and mefloquine. The first study was designed evaluate the potential interaction after multiple doses. Mefloquine significantly decreased steady- state ritonavir plasma AUC by 31% and $C_{max}$ by 36%. The second study did not alter single

dose pharmacokinetics of ritonavir. It was concluded that it safe to administer these two drugs together at the doses used in the study and that ritonavir did not influence mefloquine pharmacokinetics and mefloquine had variable effects on ritonavir pharmacokinetics (Khalig, et al., 2001).

## Ketoconazole

Ketoconazole is c/s-1-acetyl-4-(*p*-[[2-(2, 4-dichlorophenyl)-2-imidazol-1- ylmethyl) 1,3-dioxolan-4-yl] methoxy] phenyl] piperazine (Cordoba-Diaz *et al.*, 2001). The structural formula of ketoconazole is 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 broad spectrum of antifungat activity, possesses some antibacterial activity (Shuster, 1984; McGrawth and Murphy, 1991) and wide tissue distribution, but strong inhibitory effect on cyclosporin oxidase and testosterone 6β-hydroxylase activity in human (Baldwin, et al., 1995).

### 1. Chemical and Physical Properties

Chemical structure: $C_{26}H_{28}Cl_2N_4O_4$(Figure 3)

Molecular weight: 531.4

pKa : 6.51, 2.94

Solubility

In alcohol: 1 in 54 (w/v)

In water: almost insoluble

Octanol/water partition coefficient: 5400 (pH 11.8) (Dollery, 1999)

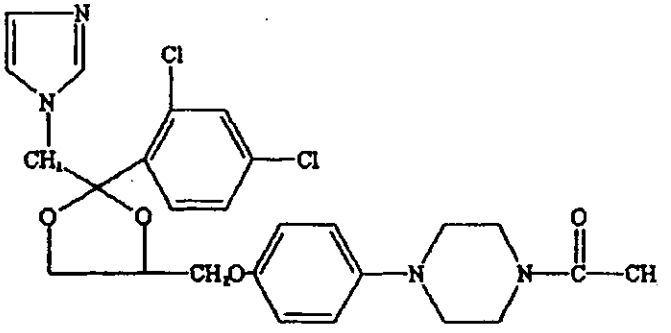



Figure 3 Structural formula of ketoconazole

## 2. Pharmacokinetics

### 2.1 Absorption

Ketoconazole is more rapidly absorbed and produces higher concentrations in plasma when administered to infants and children as a suspension than as a powder 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 ior 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 these 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(R) tablets, 1996).

### 2.2 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 Tyie, 1984). Ketoconazole is extensively bound in human whole blood (99%), with 84% to plasma proteins, largely albumin and 15% to erythrocytes; and 1% is free (Heel, et al., 1982; Chambers, 2001). Ketoconazole is highly distributed into saliva and detectable. It penetrates poorly into CSF and in the CSF of patients with fungal meningitis is less than 1% of the total drug

concentration in plasma. In study, plasma protein binding of ketoconazole was altered in patients with chronic renal disease and hepatic cirrhosis, with the percentage of free ketoconazole markedly increased compared to controls (Martinez-Jorda, et al., 1990).

2.3 Elimination

Ketoconazole is extensively metabolized by hydroxylation of the imidazole and by oxidative /V-dealkyllation of the piperazine ring dependent on microsomal enzymes in the liver. Ketoconazole itself 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(R) tablets, 2000; Graybill and Drutz, 1980). Renal excretion of ketoconazole is 13% (Prod Info Nizoral(R) tablets, 2000; Graybill and Drutz, 1980) and excreted as unchanged drug is 2% to 4% (Prod Info Nizoral(R) tablets. 2000). In three human volunteers given $^{3}$H-ketoconazole 2.5 mg/kg about 70% of the administered dose was excreted within 4 days (57% in feces and 13% in urine). Of the fecal 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, seen at higher doses of ketoconazote (Brass, et al., 1982). Renal insufficiency dose not affect the plasma concentration 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 (Daneshment, et al., 1983). With an oral dose of 200 mg the range of mean ketoconazole half-life 1.51 to 4 hours. At higher dose (400 and 800 mg) the mean half-life were 3.7 hours (range from 1.3 to 11.6 hours) (Maksymink, et al., 1982).

3. FDA Labeled Uses

3.1 African histoplasmosis

3.2 Candidiasis-chronic mucocutaneous

3.3 Candidiasis-disseminated

3.4 Candidiasis-esophageal

3.5 Candidiasis-oral

3.6 Cavitary histoplasmosis

3.7 Chromomycosis systemic infection

3.8 Coccidioidal meningitis

3.9 Coccidioidomycosis

3.10 Cutaneous dermatophyte infections

3.11 Dermatitis-seborrheic

3.12 Dermatomycosis

3.13 Disseminated Candida sepsis

3.14 Disseminated histoplasmosis

3.15 Esophageal candidiasis

3.16 Histoplasmosis

3.17 Histoplasmosis in AIDS

3.18 Mucocutaneous candidiasis

3.19 Oral candidiasis

3.20 Oral candidiasis in AIDS

3.21 Oropharyngeal candidiasis

3.22 Paracoccidioidomycosis

3.23 Pityriasis versicolor

3.24 Seborrheic dermatitis

3.25 Thrush

3.26 Tinea cruris

3.27 Tinea pedis

3.28 Tinea versicolor

4. Therapeutic use

4.1 Acne

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

4.2 Arthritis, candidal

Ketoconazole has been used to treat Candida arthritis in doses of 400 to 800 milligrams/day; however, the efficacy of ketoconazole for this indication has not been fully established (Silveira, et at.,1993).

4.3 Athlete androgen administration test

The suppressive effects of ketoconazole on testicular androgen production demonstrated an effective test to distinguish testosterone and other androgen administration in a study involving testosterone pretreated male subjects and untreated healthy male subjects. Study participants received ketoconazole 400 milligrams (mg) at 0 and 2 hours 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 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 were administered ketoconazole on day 3, the controls (n=9) and athletes that had been previously tested three times with a naturally high testosterone to epitestosterone ratio (T/EpiT) (n=5). Serum testosterone and urinary T/EpiT ratio were evaluated every two hours for an 8-hour time period. A significant

difference was evident between the testosterone treated groups and the untreated groups (p less than 0.0001). The serum testosterone concentration remained unchanged and the T/EpiT ratio increased in the testosterone treated study groups after the administration of ketoconazole, whereas the serum testosterone concentration and T/EpiT ratio decreased by 90% and 60%, respectively, after ketoconazole administration in the untreated study 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 addition, 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).

### 4.4 Blastomycosis

Infections with *Blastomyces dermatitidis* are common in the Midwest and Southeastern areas in the US. 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 system (CNS), amphotericin B is considered the drug of choice (Anon, 1992; Mandell, et al., 1990). For patients who are immunocompromised such as transplant patient or patients with AIDS, treatment with amphotericin B followed by long-term treatment with ketoconazole in doses of 400 to 800 milligrams daily has been used (Serody, et al., 1993; Pappas, et al., 1992).

4.5 Candidiasis-chronic-mucocutaneous

Clinical trials have documented the efficacy of ketoconazole in the treatment of chronic mucocutaneous candidiasis in doses of 100 to 400 milligrams daily for 1 to 7 months (Mobacken and Moberg, 1986).

4.6 Candidiasis-cutaneous

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

4.7 Candidiasis-disseminated

For the treatment of disseminated candidiasis, amphotericin B is the drug

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

4.8 Candidiasis-esophageal

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

4.9 Candidiasis-oral

Ketoconazole has been effective for the treatment of oral and esophageal candidiasis. Ketoconazole 200 to 800 milligrams/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., 19B4).

### 4.10 Candidiasis-urinary

Ketoconazole was used in 11 patients to treat 13 episodes of fungal urinary infections. Eight of the infections were caused by Candida species, 1 by mixed C. *tropicalis* and *T. glabrata,* and 3 by *T. glabrata*. Ketoconazole was administered orally at doses of 200 to 800 milligrams/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).

### 4.11 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, 1983; Kovacs, et al., 1990; Baibi, et al., 1986; Sobel, 1986).

### 4.12 Chromomycosis-systemic infections

Ketoconazole is indicated for the treatment of systemic chromomycosis infections. The usual oral adult starting dose for chromomycosis infections is ketoconazole 200 milligrams administered one time. Then the ketoconazole dose may be increased to 400 milligrams once daily. For children over 2 years old, a single daily dose of ketoconazole 3.3 to 6.6 milligrams/kilogram is recommended. Treatment should be continued for a minimum of 6 months (Prod Info Nizoral(R), 1995).

### 4.13 Coccidioidomycosis

The Medical Letter recommends either ketoconazole or amphotericin B as drugs of choice to treat Coccidioidomycosis infections. The recommended dose of ketoconazole is 400 milligrams daily (Anon, 1992).

### 4.14 Cushing's disease

Ketoconazole produced a biochemical and hormonal improvement for most patients with Cushing's syndrome secondary to ectopic adrenocorticotropin (ACTH) production by malignant tumors in a retrospective chart review. A total of 15 patients were assessable, including 11 patients with primary lung cancer (9 small cell lung cancer (SCLC), one mixed SCLC/non-SCLC. and one non-SCLC), two metastatic carcinoid tumors (pancreatic and bronchial), one metastatic hepatocellular carcinoma, and one metastatic medullary carcinoma of the thyroid. Patients received ketoconazole orally starting at 400 milligrams daily in divided doses and titrated according to 24-hour urinary free-cortisol (UFC) levels up to 1200 mg daily for a median duration of 26 days. Concurrent combination chemotherapy was administered to nine patients. Complete response (CR) was defined as normal post treatment UFC levels or normalization of morning plasma cortisol levels if the UFC levels were not available. Partial response (PR) was considered a UFC reduction to less than 50% of baseline, and all other results were defined as no response (NR). Ten of the 12 assessable patients demonstrated a hormonal response, including seven CR and three PR (median duration 25 days). Clinical and biochemical improvement occurred in most patients, including hypokalemia, metabolic alkalosis, diabetes, and hypertension. Four patients experienced hypoadrenalism (three definite and one probable) and possibly were associated with the level of hormonal control. The cortisol response to stress may be diminished in patients with good hormonal control

secondary to ketoconazole therapy, supporting the role of prophylactic replacement corticosteroids in these patients, and the administration of moderate to high-dose corticosteroids for potential stress situations. Progressive malignant disease was responsible for death in most patients (median survival 19 weeks), accompanied by worsened hypercortisolemia despite ketoconazole therapy (Winquist, et al., 1995).

### 4.15 Cutaneous dermatophyte infections

Oral ketoconazole is indicated for the treatment of cutaneous dermatophyte infections that have not responded to topical therapy or oral griseofulvin or those unable to tolerate griseofulvin. Patients received 100 to 400 milligrams oral ketoconazole daily for an average of 2 months, although 8 months of duration have been observed (Obasi and Ozoh, 1988; Hersle, 1985; Baker and Para, 1984; Hay and Clayton, 1982; Laurberg, 1982; Robertson, et al., 1982; Degreef, et al., 1981; Legendre and Steltz, 1980; Galimberti, et al., 1980; Robertson, et al., 1980; Welsh and Rodriguez, 1980).

### 4.16 Dermatitis-seborrheic

Ketoconazole 2% cream is indicated for the treatment of seborrheic-dermatitis. The manufacturer recommends application of the 2% shampoo twice a week for 4 weeks, with at least 3 days between uses, and then intermittently as needed for the treatment of scaling due to dandruff until clearing of the lesions occur (Prod Info Nizoral (R) Shampoo, 1995).

### 4.17 Histoplasmosis

Ketoconazole may be used to treat less serious histoplasmosis infections, but for infections in immunocompromised host or for serious infections, amphotericin B should be used. For patients with AIDS long-term suppressive therapy is necessary to prevent recurrence. An oral azole (such

as ketoconazole, itraconazole, or fluconazole) or amphotericin B in a weekly or biweekly schedule has been used for maintenance therapy (Neubauer and Bodensteiner, 1992). Ketoconazole 400 milligrams daily for 12 months has been recommended in adults with progressive disseminated histoplasmosis (Hawkins, et al., 1981).

### 4.18 Ovarian hyperandrogenism

Ketoconazole has been recommended to be used only in select patients with non-tumoral hyperandrogenism because of adverse effects. In a study of 37 women with hirsutism, acne and oligomenorrhea, low-dose ketoconazole (400 milligrams/day) was administered for 9 months. Overall drop-out rate due to adverse effects was 30%, with 9 patients discontinuing therapy, particularly because of dyspepsia or abnormal menstrual bleeding. Hirsutism was improved in all 26 patients who completed the treatment course. Acne was markedly improved after 3 months of treatment. In addition, there were significant decreases in androgenic steroids with concurrent increases in estradiol (Vidal-Puig, et al., 1994).

### 4.19 Paracoccidioidomycosis

Available studies have demonstrated the efficacy of ketoconazole in the treatment of paracoccidioidomycosis in Latin America (Cuce, et al., 1980; Negroni, et al., 1980; Restrepo, et al., 1980). Ketoconazole (in a dose of 200 to 400 milligrams daily) or amphotericin B is considered the drugs of choice for the treatment of paracoccidiomycosis infections (Anon, 1992).

### 4.20 Transplantation

Ketoconazole may help reduce the incidence of graft rejection (Sobh, et al., 1995).

5. Untoward Effects.

The most common side effects of ketoconazole are dose-dependent nausea, anorexia, and vomiting which occur in approximately 10% of patients receiving 400 mg/day but increase to more than 50% in patients receiving more than 800 mg/day (Chambers, 2001; Sugar, et al., 1987). Administration of ketoconazole with food at bedtime, or in divided dose may improve tolerance. An allergic rash occurs in about 4% of ketoconazole-treated patient and pruritus without rash in about 2%. Hair loss has also been reported.

Ketoconazole inhibits steroid biosynthesis in patients, as it does in fungi, by inhibition of cytochrome P450-dependent enzyme systems. Several endocrinologic abnormalities thus may be evident. Approximately 10% of females report menstrual irregularities. A variable number of males experience gynecomastia and decrease libido and potency. At high doses, azoospermia has been reported, but sterility has not been permanent. Doses of ketoconazole as low as 400 mg can cause a transient drop in the plasma concentrations of free testosterone and estradiol C-17p. Similar doses of 800 to 1200 mg of ketoconazole have been used to suppress plasma cortisol in patients with Cushing's disease. Similar doses were evaluated in patients with prostatic carcinoma. Hypertension and fluid retention have been reported and are associated with elevated concentrations of deoxycorticosterone, corticosterone, and 11-deoxycortisol. Although reports of Addison's disease due to ketoconazole are not convincing, it would seem prudent to discontinue the drug before major surgical procedures and to avoid using high doses in patients with trauma, severe bums, of other stressful conditions.

Mild, asymptomatic elevation of aminotransferase activity in plasma is common occurring in 5% to 10% of patients; these values revert to normal spontaneously. Symptomatic drug induced hepatitis is rare but is potentially

fatal. Hepatitis may occur after a few days of treatment, or it may be delayed for many months. The earliest symptoms are anorexia, malaise, nausea, and vomiting, with or without dull abdominal pain. Liver function tests usually mimic the pattern seen with hepatitis A. but a cholestatic or mixed picture can occur. Patients should be alerted to the symptoms and asked to return for liver function tests should this toxicity be suspected. Ketoconazole is teratogenic in animals, causing syndactyly in rats. Its use during pregnancy is not recommended, and because of secretion of the drug into breast milk, its use in nursing mothers also is unwise (Chambers, 2001).

6. Contraindication

A. Hypersensitivity to ketoconazole.

B. Do not use for treatment of fungal meningitis because it penetrates poorly into the CSF.

C. Concurrent use with astemizole [Enzyme inhibiting drugs such as ketoconazole may lead to high levels 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)] terfenadine [Concomitant use of terfenadine and ketoconazole is contraindicated (Prod Info Nizoral(R), 1998). Co-administration may result in QT prolongation due to inhibition of terfenadine metabolism (Mathews, et al., 1991; Eller, et al., 1991; Honig, et al., 1993)], cisapride [Concomitant administration of cisapride and ketoconazole has resulted in marked increases in cisapride plasma concentrations and prolonged QT interval (Pers Comm, 1995)].

7. Precautions

7.1 Impaired hepatic function

7.2 Impaired adrenal reserve

7.3 High-dose of ketoconazole therapy. Ketoconazole therapy may precipitate adrenal insufficiency, especially in patients with impaired stress response (Khosla, et al., 1989).

7.4 Patients with achlorhydria

## 8. Drugs Interaction

### 8.1 Oral Anticoagulants

A patient had been treated with warfarin for three years for a pulmonary embolism, and later received ketoconazole 200 mg twice daily for chronic vaginal thrush infection. After three weeks of treatment 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 stopped, warfarin dosage reduced. Over the next three weeks her warfarin control was restabilised 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.

### 8.2 Benzodiazepines

Chlordiazepoxide is extensively oxidized in the liver with little urinary excretion of the parent drug. Ketoconazole impaired Chlordiazepoxide clearance from plasma. After a single dose of ketoconazole there was a 20% decrease in clearance and 26% decrease in volume of distribution 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-desmethylchlordiazepoxide. 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 result in increased serum concentrations of alprazolam and associated alprazolam toxicity (excessive sedation, fatigue, ataxia, slurred speech, 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 (Prod Info Xanax(R), 1997).

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. Triazolam commonly causes amnesia. 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 itraconazole increased 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 pharmacokinetics 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).

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 (Oikkola, et al., 1994). Psychomotor tests and subjective reporting of drowsiness with the combination indicated significant increases in sedative effects. Oral midazolam is not recommended for patients receiving ketoconazole. Ketoconazole is a 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(R), 1997). Inhibited CYP3A activity caused by ketoconazole appears to be greater in the intestine than in the liver (Tsunoda, et al., 1999).

### 8.3 Calcium channel blocking agents

Ketoconazole inhibits hepatic cytochrome isoenzyme CYP3A4 (Gibaldi, 1992; Prod Info Nizoral(R). 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 treament with nisoldipine 5 mg alone (Heinig, et al., 1999). Literature reports have documented substantial peripheral edema and/or elevated calcium antagonist serum concentrations during concurrent use of itraconazole and felodipine, isradipine, or nifedipine (Neuvonen and Suhonen, 1995; Tailor, et al., 1996). Since the other triazole and imidazole antifungals also inhibit CYP3A4, this interaction would be expected to occur with other combinations.

### 8.4 Amphotericin B

Animal studies and in vitro investigations have found antagonism between amphotericin B and azole antifungal derivatives. The mechanism of action of azoles is to 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(R), 1999).

### 8.5 Tirilazad

Tirilazad mesylate is a membrane lipid peroxidation inhibitor that shows efficacy in reducing the damaging effects of lipid peroxidation on the cell membrane triggered by brief periods of ischemia. Tirilazad 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 tirilazad mesylate AUC by 67% and 309% for intravenous and oral administration, respectively. Mean AUC for active metabolite of tirilazad (U-89678) were increased 472% and 720% by ketoconazole administration with iv and oral tirilazad, respectively, whereas increases of more than 10-fold in mean LI-87999 (another active metabolites) AUC. Ketoconazole increased the bioavailability 20.9% by decreasing the first-pass liver and gut wall metabolism of tirilazad 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).

### 8.6 Quinine

Mirghani et al. (1999) showed the effect of ketoconazole on quinine pharmacokinetics, it (which inhibits CYP3A4) significantly decreased the mean apparent oral clearance of quinine by 31%, whereas co-administration with fluvoxamine (which inhibits CYP1A2 and to some extent CYP2C19) had no significant effect on the mean apparent oral clearance of quinine. Co-administration of ketoconazole also decreased the mean AUC of 3-hydroxyquinine, whereas co-administration with fluvoxamine increased 3-hydroxyquinine AUC significantly.

CYP3A4 is important for the 3-hydroxylation of quinine *in vivo*. On the other hand, CYP1A2 had no significant effect on this metabolic pathway.

### 8.7 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 $2^{nd}$ day of a 5 days regimen of 200 mg ketoconazole once daily in a crossover design. Ketoconazole increased R, R (-) - reboxetine and S, S (+)-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 hours) was significantly longer than after reboxetine alone (14.8 and 14.4 hours; $P < 0.005$). The AUC ratio for R, R (-)-reboxetine to S, S (+)-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).

### 8.8 Amprenavir

Twelve individuals received single doses 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 (Prod Info Agenerase(R), 2000). The significance of this interaction is unknown, but unlikely to be clinically important (Polk, et al., 1999).

### 8.9 Tolbutamide

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

### 8.10 Antihistamine Drugs

Enzyme inhibiting drugs such as ketoconazole may lead to high levels 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 concentrations resulting in cardiac toxicity, the manufacturer warns that astemizole use with ketoconazole is contraindicated (Anon, 1993; Prod Info Hismanal(R), 1998).

### 8.11 Antacids

Ketoconazole can be administered with some antacids that could modify its dissolution rate and reduce its absorption leading to therapeutic failures (Dýaz, et al., 2001). Concurrent administration of ketoconazole with Maalox(R), cimetidine or sodium bicarbonate has resulted in both a decrease in plasma peak concentration and the AUC of ketoconazole (Brass, et al., 1982; Carison, et al., 1983).

### 8.12 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 increases 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).

### 8.13 Oestrogen

Oestrone undergoes extensive oxidative metabolism by cytocrome P450 enzymes. A major route is hydroxylation. The hydroxylation of oestrone are 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 oestrone (+4029 nmol $l^{-1}$ h) and its $C_{max}$ (+306 nmol $l^{-1}$).

### 8.14 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 (Campbell and Carroll, 2001). In contrast, studies in primates and humans have produced conflicting results using cortisol synthesis inhibitors for attenuating cocaine-related behaviors and subjective effects. To explore the treatment implications of these findings, 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 trial. 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 trial, 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., 2001).

### 8.15 Cyclosporine

Five male renal allograft patients of mean age 22 ± 2.3 years displayed a mean AUC of cyclosporine before starting ketoconazole to be 1581.8 ± 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).

## 9. Factors Affecting the Pharmacokinetics of Ketoconazole

### 9.1 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).

### 9.2 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; Bennett, et al., 1987).

### 9.3 Hepatic Insufficiency

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

## Cytochrome P-450 System

### 1. Introduction

Drugs are mainly metabolized by enzymes in the liver, kidneys, gastrointestinal tract, skin, and lungs (Benet, et al., 1996). Drug-metabolizing enzymes are found in the endoplasmic reticulum of cells in these tissues and are classified as microsomal enzymes. There are 2 types of drug-metabolizing enzymes: phase I enzymes, or mixed function oxidases, which catalyze predominantly oxidation, reduction, and hydrolysis; and phase II enzymes, which catalyze glucuronidation, sulfation, or acetylation (Renton, 1986).

The majority of phase I metabolism is catalyzed by the cytochrome P-450 enzymes (CYP), which are heme containing, membrane-bound proteins. These enzymes, found at highest concentration in the hepatocytes, biotransform lipophilic drugs to more polar compounds that can be excreted by the kidneys (Spatzenegger and Jaeger, 1995). The metabolites are usually less active than the parent compound, although some drugs undergo biotransformation to pharmacologically active agents. In some cases the metabolites can be toxic, carcinogenic, or teratogenic (Renton. 1986).

CYP represents a superfamily of enzymes. They are found in animals, plants, yeast and bacteria. In mammals, some CYP are involved in pathways of steroid biosynthesis and do not metabolize foreign compounds. However, the vast majority of these enzymes, the foreign compound metabolizing CYP, appear to oxidize chemicals that are not normal constituents of the body. CYP are named with the root CYP followed by an Arabic number and upper case letter designating the family and subfamily, respectively. Individual CYP forms are denoted by Arabic number that follows the subfamily letter (Gonzalez and Idle, 1994).The cytochrome P450 proteins are embedded in the lipid bilayer of

the smooth endoplasmic reticulum. An important associated protein, NADPH-cytochrome P450 reductase, is also attached to this lipid bilayer in a stoichiometry of about ten P450 molecules to one reductase (Benet, et al., 1991). A simplified scheme of the oxidative cycle is presented in Figure 5. Briefly, oxidized ($Fe^{3+}$) cytochrome P450 combines with a drug substrate to form a binary complex (step 1). NADPH donates an electron to the flavoprotein reductase, which in turn reduces the oxidized cytochrome P450-drug complex (step 2). A second electron is introduced from NADPH via the same flavoprotein reductase, which serves to reduce molecular oxygen and to form an "activated oxygen- cytochrome CYP-substrate" complex (step 3). This complex in turn transfers "activated" oxygen to the drug substrate to form the oxidized product (step 4) (Correia, 1998).

2. Human Hepatic Cytochrome P450s (P450s)

The CYP comprise a superfamily of haemoproteins which contain a single iron protoporphyrin IX prosthetic group. This superfamily is subdivided into families and subfamilies that are defined solely on the basis of amino acid sequence homology. To date, at least 14 CYP gene families have been identified in mammals. The mammalian CYP families can be functionally subdivided into 2 major classes, those that involve the biosynthesis of steroids and bile acids and those that primarily metabolize xenobiotics. Three main CYP gene families, CYP1. CYP2 and CYP3 are responsible for most hepatic drug metabolism. Although the CYP1 and CYP3 gene families are relatively simple (i.e. CYP1A, CYP1B and CYP3A), the CYP2 gene family is comprised of many subfamilies (e.g., CYP2A, CYP2B, CYP2C, CYP2D, CYP2E, etc). These isoforms have the same oxidizing center (the haem iron), but differ by their protein structures (Lin and Lu, 1998).

For different CYP, specificity control is governed by the entry of the substrate into the active site and the direct interaction of amino acids in the active site with the substrate. Because the interaction of substrate and mammalian CYP generally lacks absolute complementarily, substrates often bind to the enzyme active site in several different configurations, resulting in multiple metabolites with regio-and stereospecificity unique to each isoform.

In general, a significant drug-drug interaction occurs only when 2 or more drugs compete for the same enzyme and when the metabolic reaction catalyzed by this enzyme is major elimination pathway. Drug-drug Interactions can also occur when the CYP responsible for the metabolism of a drug is induced by long -term treatment with an other drug. Thus, definitive assessment of role of an individual CYP in a given metabolic pathway is essential in determining and predicting the potential for drug interaction. To identify which CYP isoforms are responsible for the oxidative metabolism of drugs, a general strategy has emerged for *in vitro* study. This involves: (a) use of selective inhibitors; (b) immunoinhibition; (c) catalytic activity in cDNA-based vector system; (d) catalytic activity in purified enzymes; and (e) metabolic correlation of activity with markers for known CYP isoforms. Each approach has its advantages and disadvantages, and a combination of approaches is usually required to accurately identify the CYP isozyme responsible for the metabolism of a given drug (Lin and Lu. 1998).

### 3. Mechanisms of Inhibition of CYP

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

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

(ii) reduction of the haem group from the ferric to the ferrous state by

an electron provided by NADPH via CYP reductase

(iii) binding of molecular oxygen

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

(v) cleavage of the 0-0 bond

(vi) substrate oxygenation

(vii) product release.

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

The mechanisms of CYP inhibition can be divided grossly into 3 categories: reversible inhibition, quasi-irreversible inhibition and irreversible inhibition. Among these, reversible inhibition is probably the most common mechanism responsible for the documented drug interactions (Halpert, 1995).

### 3.1 Reversible Inhibition

Many of the potent reversible CYP inhibitors are nitrogen-containing drug, including imidazoles, pyridines and quinolines. These compounds cannot only bind to the prosthetic haem iron, but also to the lipophilic region of the protein. Inhibitors that simultaneously bind to both regions are inherently more potent inhibitors. The potency of an inhibitor is determined both by its lipophilicity and by the strength of the bond between its nitrogen lone electron pair and the prosthetic haem iron. For example, both ketoconazole and cimetidine are imidazole-containing compounds that interact with ferric CYP at its sixth axial ligand position to elicit a type II optical difference spectrum. The coordination of a strong ligand to the pentacoordinated iron, or the displacement of a weak ligand from the hexacoordinated haem by a strong ligand, gives rise to a "type II" binding spectrum. However, cimetidine is a

relatively weak reversible inhibitor of CYP, an apparent result of an intrinsic low binding affinity to microsomal CYP. This letter property is most probably because of the low lipophilicity of cimetidine (logP=0.4). On the other hand, ketoconazole, a potent CYP inhibitor, has a high lipophilicity (logP=3.7). Similarly, fluconazole contains a triazole that binds to the prosthetic haem iron but is a weak reversible CYP inhibitor, again due mainly to its low lipophilicity.

Many antimalarial agents (such as primaquine. chloroquine. amodiaquine and mefloquine) contain a quinoline ring and are potent reversible CYP inhibitors. However, the inhibition activity is not associated with the quinoline structure, since the pyridine nitrogen is sterically hindered. Instead, the amino group in substituents on the quinoline ring appears to be the primary determinant of the observed inhibition potency. The terminal amino group in the 8-substituent of primaquine is believed to be involved in the direct binding to the haem iron of the ferric CYP (Lin and Lu, 1998).

### 3.2 Quasi-Irreversible Inhibition via Metabolic Intermediate Complexation

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

MI complex is so stable that the CYP involved in the complex is unavailable for drug metabolism, and synthesis of new enzymes is the on means by which activity can be restored. The nature of the MI complexation is, therefore, considered to be quasi-irreversible (Lin and Lu, 1998).

### 3.3 Irreversible Inhibition of CYP

Drug containing certain functional group can be oxidized by CYP to reactive intermediates that cause irreversible inactivation of the enzyme prior to its release from the active site. Because metabolic activation is required for enzyme inactivation, these drugs are classified as mechanism-based inactivators or suicide substrates. The mechanism-based inactivation of CYP may result from irreversible alteration of haem or protein, or a combination of both. In general, modification of the haem group invariably inactivates the CYP, whereas protein alteration will result in loss of catalytic activity only if essential amino acids, which are vital for substrate binding, electron transfer and oxygen activation, are modified.

## 4. Mechanism of Induction of CYP

One of the intriguing aspects of the CYP is that some of these enzymes, but not all, are inducible. Human CYP1A1, CYP2C9, CYP2E1 and CYP3A4 are known to be inducible. Unlike CYP inhibition, which is an almost immediate response, CYP induction is a slow regulatory process that can reduce drug concentrations in plasma, and may compromise the efficacy of the drug in a time-dependent manner. Unless care is taken in study design, the pharmacokinetic and clinical consequences of CYP induction are often overlooked in clinical studies.

Although the phenomenon of CYP induction has been known for more than 4 decades, only in recent years we have begun to uncover the mechanisms involved in induction. From a biological point of view, induction is

an adaptive response that protects the cells from toxic xenobiotics by increasing the detoxification activity. While in most cases CYP induction is the consequence of an increase in gene transcription, some nontranscriptional mechanisms also are known to be involved.

For many years, scientists have been trying to solve the mystery of how the cells recognize the inducing agents and how the signal is transferred to the transcriptional machinery. With the exception of the CYP1A1 isoform, the molecular mechanisms involved in CYP induction are still not fully understood. In the case of CYP1A1, inducing agents bind to cytosolic polycyclic aromatic hydrocarbon (Ah) receptors and are translocated into the nucleus. The transcriptional process include a sequence of events: ligand-dependent heterodimerisation between the Ah receptor and an Ah receptor nuclear translocator, interaction of the heterodimer with a xenobiotic-responsive enhancer, transmission of the induction signal from the enhancer to a CYP1A1 promoter, and alteration in chromatin structure. This is followed by subsequent transcription of the appropriate mRNA and translation of the corresponding proteins.

In drug therapy, there are 2 major concerns related to CYP induction. First, induction will result in a reduction of pharmacological effects caused by increased drug metabolism. Secondly, induction may create an undesirable imbalance between "toxification" and "detoxification". Like a double-edged sword, induction of drug metabolizing enzymes may lead to a decrease in toxicity through acceleration of detoxification, or to an increase in toxicity caused by increased formation of reactive metabolites. Depending upon the delicate balance between detoxification and activation, induction can be a beneficial or harmful response (Lin and Lu, 1998).

## 5. Clinical Implications

### 5.1 Inhibition of CYP

The clinical relevance of drug inhibition will depend on a number of considerations. One of the most important considerations is the therapeutic index of the drug. Patients receiving anticoagulants, antidepressants or cardiovascular drugs are at a much greater risk than patients receiving other kinds of drugs because of the narrow therapeutic index of these drugs. Although most interactions that can occur with these agents are manageable, usually by appropriate dosage adjustment, a few are potentially life threatening.

For example, co-administration of terfenadine, an antihistamine agent, and ketoconazole led to fatal ventricular arrhythmias in some patients. Terfenadine is widely used histamine $H_1$ receptor antagonist. It is metabolized extensively by CYP3A4 in human to form 2 metabolites by /V-dealkylation and hydroxylation. After oral administration of a 60 mg dose, terfenadine is usually undetectable in plasma because of extensive first pass metabolism. Concurrent administration of drugs that inhibit terfenadine metabolism can result in an excessive increase in plasma concentration of terfenadine.

Clinical data showed that itraconazole and erythromycin also impair the metabolism of terfenadine. Because CYP3A4 represents a major CYP isoform in human liver, and because CYP3A4 has a broad spectrum of substrate specificity, it is likely that many other drugs are capable of inhibiting terfenadine metabolism. Because of its undesirable properties, terfenadine was recently withdrawn from sale or had its use restricted in several countries. Inhibition can also reduce clinical efficacy, if the drug is a prodrug requiring metabolic activation to achieve its effects and activation is blocked.

Reversible enzyme inhibition is transient; the normal function of CYP enzymes continues after the inhibitor has been eliminated from the body. It contrast, the loss of enzyme activity caused by irreversible inactivation persists even after elimination of inhibitor, and *de novo* biosynthesis of new enzymes is the only means by which activity can be restored. Clearly, clinical and pharmacokinetic consequences of irreversible drug inhibition are quite complicated, depending on the duration and frequency of administration. The long-term effects of irreversible inhibition on CYP are yet unknown, and further studies need to address this question.

Metabolic drug interaction is usually regarded as potentially dangerous, or at least undesirable. However, there are times when these interactions may be exploited. For example, because these 2 drugs are substrates for the same human CYP3A4, the antifungal agent ketoconazole is used with cyclosporin, an immunosuppressive agent, to prolong the elimination of the cyclosporin. The idea is to use the relatively inexpensive ketoconazole to specifically inhibit the metabolism of the very expensive cyclosporin, thereby minimizing the cost of long-term immunosuppressive therapy. Keogh, et al. (1995) have reported that ketoconazole reduced by 80% the dose of cyclosporin needed to maintain target concentrations in patients after cardiac transplantation, with a cost savings per patient of approximate $US 5200 in first year.

### 5.2 Induction of CYP

Usually, metabolites are less pharmacologically active than the parent drug and, therefore, enzyme induction results in a reduction in pharmacological effect because of increased drug metabolism. In some cases, the metabolites formed during biotransformation may be chemically reactive, so that enzyme induction may result in increased toxicity caused by the increased production of the toxic metabolites.

Rifampicin is one of the most potent enzyme inducers known to humans. It induces several CYP isoforms, including CYP2C and CYP3A. Clinical studies in healthy volunteers demonstrated a reduction in the thrombin time and a corresponding decrease in the plasma half-life of warfarin following treatment with rifampicin (Lin and Lu. 1998).

Another clinically important interaction with rifampicin involves the concomitant administration of oral contraceptives, which has been reported to result in menstrual disturbance and unplanned pregnancies. The increased metabolism of both estrogenic and progesterogenic components of oral contraceptives is believed to be the underlying mechanism.

Although enzyme induction generally reduces the pharmacological effect because of increased drug metabolism, sometimes the formed metabolites has the same pharmacological activity as the parent drug. Thus the clinical consequences of enzyme induction will be determined by the relative reactivity of the parent drug and the formed metabolite. During concomitant administration of inducers, increasing the drug dosage can circumvent the reduction in drug concentration. However, if dosages are increased, there is a danger of excessive accumulation of drug when the inducer is withdrawn and enzyme activity returns to normal (Lin and Lu, 1998).

## P-Glycoproteins

### 1. Introduction

Active transport of drugs and their metabolites has been recently recognized as an important issue in pharmaceutics. Numerous transporters have been characterized in the liver, kidney, intestine, and lung, which serve diverse functions including ion, sugar, amino acid, and peptide transport as well as drug and metabolite disposition. This section focuses on one of these transporters, P-glycoprotein (P-gp), an adenosine triphosphate (ATP) dependent drug transporter that has been extensively characterized for its role in multidrug resistance in cancer chemotherapy. Expression of this protein in tumors is associated with decreased intracellular accumulation of cytotoxic drugs, thereby enhancing cell survival in the presence of otherwise cytotoxic drug levels. P-gp is promiscuous in its ability to interact with a large number of structurally and mechanistically distinct drugs, resulting in tumors that are cross-resistant to a diverse number of drugs, hence term multidrug resistance (Silverman, 2000).

One physiologic role of P-gp is to serve as a barrier to entry and as an efflux mechanism for xenobiotics and cellular metabolites. It has also been suggested that P-gp may limit intestinal drug absorption to constrain oral drug bioavailability. Since the discovery of the drug efflux activity of P-gp, numerous investigations have attempted to inhibit P-gp-mediated drug efflux with the ultimate goal of increasing the efficacy of cancer chemotherapy. Initial attempts used existing compound: however, because of undesirable pharmacologic activities or limited success, ongoing investigations are using novel agents that are more specific and potent (Silverman, 2000).

Recognition that P-gp is a critical determinant of oral drug bioavailability has generated an additional application for P-gp reversal. This section focuses on the role of P-gp in drug absorption and disposition and the potential consequences of drug interactions between substrates and/of inhibitors of this protein. This section briefly discusses the salient features of this transporter; for detailed information on the biology and molecular characterization of P-gp, refer to one of the numerous excellent reviews on this protein and its gene family (Silverman, 2000).

2. MDR Gene Family

P-gps are encoded by members of a small gene family referred to as the multidrug resistance (MDR) genes. Because of alternative naming schemes that evolved from the independent laboratories that isolated each of the cDNAs, the nomenclature of the MDR genes can be confusing. Humans and other primates have two members of this gene family, MDR1 and MDR2 (alternatively referred to as MDR3), whereas mice, hamsters, and rats have three *(mdr1a, mdr1b and mdr2)*. The MDR1 gene encodes a drug transporter that is capable of conveying resistance to a large number of compounds. In rodents, two genes the mdr1a *(pgp1. mdr3)* and *mdr1b (pgp2. mdr1)* correspond to the human MDR1 and encode drug transporters. In contrast, the MDR2 gene encodes a phospholipid transporter, which is not involved in drug absorption or disposition and is not discussed herein (Silverman, 2000).

3. Structure of P-glycoprotein

MDR1 is a large gene spanning more than 100 kb on chromosome 7, with 28 exons that are spliced into a 4.5-kb mRNA. The encoded P-gp is an integral membrane protein with a molecular weight of approximately 170 kDa. P-gp functions as an energy-dependent membrane pump, which extrudes generally cationic or neutral, hydrophobic drugs from cells.

P-gp is a member of the large, ATP-binding cassette (ABC) transporter family. Hundreds of these traffic ATPases have been identified in bacteria, plants, fungi, and animal cells and are important in the movement of a large number of nutrients and waste products. ABC transporters transport virtually any class of substrate, including ions, sugars, amino acids, peptides, and polysaccharides. These membrane transporters typically have four domains: two have up to six membrane-spanning regions and two, locate at the cytoplasmic surface, bind ATP and couple its hydrolysis to substrate transport. Most notably in prokaryotes, these individual domains are encoded by separate genes; however, in mammals, they are often encoded by a large single gene such as MDR1. Examples of ABC transporters include the *Escherichia coli* MalEFGK gene, which imports maltose, the *Saccharomyces cervisiae* STE6, which exports the peptide a-mating factor, and the *Plasmodium falciparum* transporter *pfrndr,* which transports chloroquine and mediates drug resistance.

Sequence analysis revealed that P-gp is made up of 1,280 amino acids with roughly bilateral symmetry; the amino and carboxy halves of the protein each have six transmembrane domains and an ATP-binding region. This structural model for P-gp has been investigated using antibody mapping, site-directed mutagenesis, and biochemical analysis. Mapping epitope domains with MRK-16, an antihuman monoclonal antibody, demonstrated that the first and fourth predicted loops are extracellular. Similarly, antipeptide antibodies to $Glu^{398}$-$Lys^{408}$ and $Leu^{1206}$ -$Thr^{1226}$ recognize their epitopes in permeabilized, but not intact, cells, confirming their predicted intracellular location mapping of the topology of cysteine residues into putative intracellular or extracellular loops provided further support for the 12-transmembrane domain model. Rosenberg and co-workers (1997) used high-resolution electron microscopy

to present a model for P-gp that is consistent with the available immunologic and biochemical analysis. At 2.5- nM resolution, P-gp appears to function as a monomer and have a 5-nM central pore, which is closed on the cytoplasmic surface of the plasma membrane forming an aqueous compartment. Two 3-nM intracellular lobes were observed and are consistent with the predicted 200-amino acid nucleotide binding domains. These data agree with the hypothesis that substrate binding and cross-linking agents interact at the cytoplasmic face of the membrane. Biochemical analysis using nickel-chelate chromatography has also suggested that P-gp functions as a monomer. Sonveaux and colleagues (1996) examined the secondary and tertiary structure of P-gp using attenuated total reflection Fourier transform infrared spectroscopy. The secondary structure of P-gp was found to contain 32% a-helix, 26% p-sheet, 29% turns, and 13% random coil; no significant alterations in these parameters occurred upon binding of verapamil, ATP, or a nonhydrolyzable ATP analogue (Silverman, 2000).

4. Function of P- glycoprotein

Unlike typical ABC transporters, which have a narrow, usually single, substrate range, a defining characteristic of P-gp is its ability to transport literally hundreds of compounds. Increased expression of P-gp is associated with the multidrug-resistant phenotype in which cells become cross resistant to structurally and mechanistically distinct cytotoxic drugs. Demonstration that this protein is responsible for this phenotype comes most clearly from gene transfer experiments. Transfection of high- molecular weight DNA isolated from drug-resistant cells confers a multidrug-resistant phenotype to previously drug-sensitive cells. Similarly, transfection of either the murine *mdrl* or human MDR1 cDNAs into drug-sensitive ceils also results in a 200-fold increase in resistance to daunomycin and cross resistance to adriamycin, colchicine, vincristine, and vinblastine. The level of drug resistance in MDRI-

transfected cells correlates with the expression of P-gp. Thus, transfer of the cDNA-encoding P-gp is in itself sufficient to confer a drug-resistant phenotype upon drug-resistant cells (Silverman, 2000).

5. Role of P-glycoprotein in Drug Absorption and Disposition

The role of P-gp in cancer chemotherapy is well established; however, recognition of its role in drug absorption, disposition, and potential drug interactions is more recent. P-gp can affect drug levels in several ways. For example, P-gp is expressed on the biliary canalicular membrane of hepatocytes facilitating the excretion of drugs, metabolites, and xenobiotics into the bile. Similarly, because of its expression on the apical surface of intestinal villus enterocytes, P-gp is well situated to affect the absorption of substrate drugs. A role for P-gp in detoxification pathways and limiting uptake of drugs and xenobiotics has long been postulated and has recently been substantiated by experimental observations using both *in vitro* and *in vivo* model systems.

A major contribution among the many models used to investigate the role of P-gp in drug absorption and disposition was the development of knockout mice in which the *mdr1a* alone or both the *mdr1a* and *mdr1b* genes have been functionally disrupted by homologous recombination. Using these mice, several studies have demonstrated a clear role for P-gp in the pharmacokinetics of drugs such as vinblastine, taxol, digoxin, and several cationic compounds. Mice lacking *mdr1a* exhibit reduced fecal elimination of vinblastine, digoxin, taxol, tri-n-butylmethylammonium (TbuMA), and azidopro-cainamide methoiodide (APM). These mice also exhibit increased accumulation of drugs in the liver, brain, and gall bladder, tissues, which normally express P-gp. The serum terminal half-life of intravenously administered vinblastine was longer in the knockout mice than in wild-type

animals, 3.6 versus 2.1 hours, respectively, and the fecal elimination was reduced from 20% to 25% to 9%. Vinblastine also accumulated in the brain, heart, and liver of the *mdr la-deficient* animals. Similarly, reduced fecal and intestinal elimination and increased tissue accumulation of digoxin was observed in these animals. Thus, P-gp contributes substantially to the elimination of substrate drugs through both hepatic and intestinal secretion.

The *mdr1a* knockout mice have also been used to demonstrate a clear role of P-gp in drug absorption. Increased bioavailability and altered tissue distribution was observed for paclitaxel, loperamide, vinblastine, ivermectin, cyclosporin A (CsA), human immunodeficiency virus (HIV) protease inhibitors, TBuMA and APM. Marked increases in accumulation of these drugs were observed in the brain, liver, intestine, and other tissues of the knockout versus wild-type animals. Oral administration of loperamide resulted in plasma levels that were two to three times higher in *mdr1a* knockout mice compared to wild-type mice. The lethal dose to wild-type mice was approximately 80 mg/kg, whereas, in the *mcdr1*a-deficient mice, the lethal dose was 10 mg/kg. The knockout mice had clear central opiate effects, which were absent in the wild-type mice because of the low amount of this drug that normally crosses the blood-brain barrier. Similarly, a six-fold increase in the area under the plasma concentration versus time curve (AUC) and a 11-fold increase in $C_{max}$ for orally administered paclitaxel was observed in the *mcdr1*a-deficient mice compared to the control animals. Consequently, the oral bioavailability of paclitaxel increased from 11% in wild-type mice to 35% in the knockout animals. Co-administration of the P-gp inhibitors PSC 833 or CsA with paclitaxel in wild-type animals resulted in a 10-fold increase in AUC, further supporting a role for P-gp in oral drug absorption. These data also clearly demonstrate the consequences of inhibition of P-gp on the pharmacokinetics of a co-

the intestine suggests complementary roles that may limit drug absorption and increase disposition. Another potential function for P-gp in the intestine may be to transport compounds back into the lumen. This would establish a cyclic pathway for drugs as they transit the intestine, thereby increasing the exposure time of drugs to drug-metabolizing enzymes (e.g., CYP3A) to act. The cooperative nature of CYP3A and P-gp presents a unique opportunity to affect substrate absorption and a significant potential for drug interactions (Silverman, 2000).

## 6. Drug Interactions with P-glycoprotein

### P-Glycoprotein and the Antifungal Agents

Much of the data on the effects of the antifungal agent on P-glycoprotein function comes from *in vitro* cell culture models of directional drug transport or ex *vivo* models of intestinal drug secretion. The results obtained seem to vary with the model used, making a definitive conclusion difficult. In addition, the *in vivo* significance of the $IC_{50}$ values for inhibition of *in vitro* drug transport is not clear, because estimates of P-glycoprotein-available concentrations of substrates and inhibitors in vivo are not established (Venkatakrishnan, et al., 2000).

*In vitro* transport studies suggest that ketoconazole is not a P-glycoprotein substrate, although *in vivo* studies in *mdr1a* (-/-) knockout mice have yielded conflicting results. Ketoconazole is an inhibitor of P-glycoprotein on the basis of its ability to reverse vinblastine and doxorubicin resistance, and enhance uptake and retention of the P-glycoprotein substrate rhodamine 123, in the multidrug-resistant KB-V1 human cancer cell line at concentrations of 1 to 10 mg/L. Ketoconazole also; (I) potently inhibited the basolateral-to-apical flux of the P-glycoprotein substrate digoxin across the P-glycoprotein-expressing MDCK cell line (a canine kidney cell line that has been proposed

as a model of renal tubular secretion), with an $IC_{50}$ value of approximately 2 μmol/L; completely inhibited the polarized transport of digoxin in Caco-2 cells at a concentration of 10 μmol/L and displaced verapamil (a high-affinity P-glycoprotein ligand) from P-glycoprotein preparations using Caco-2 cells, with a $K_1$ value of 1.2 μmol/L ($IC_{50}$ 13 μmol/L) in competition binding experiments, However, an $IC_{50}$ value of 119 μmol/L has been reported for inhibition by ketoconazole of the polarized transport of the P-glycoprotein/CYP3A peptidomimetic substrate K02 in MDR1-MDCK cells. In addition, the transport of rhodamine 123 by Caco-2 cells and across everted rat ileum, and the *in vivo* exsorption clearance of rhodamine 123 in rats, was only weakly inhibited by ketoconazole -25 to 40% inhibition at a ketoconazole concentration of 100 μmol/L. Although ketoconazole is a potent P- glycoprotein inhibitor in some systems, the results can vary depending on the *in vitro* model used and/or the P-glycoprotein substrate used in the assay (Venkatakrishnan, et al., 2000).

Itraconazole may be a P-glycoprotein substrate, as shown by its increased brain accumulation in *mdr1a* (-/-) mice deficient in P-glycoprotein. Itraconazole causes a concentration-dependent reversal of daunorubicin resistance in P-glycoprotein overexpressing multidrug-resistant murine cancer cells, with complete reversal at a concentration of 2.5 mg/L. Reversal by Itraconazole 0.5 to 2 mg/L of doxorubicin and etoposide resistance in human leukaemic cells has also been described. In addition, itraconazole 4.25 μmol/L increased the accumulation of vincristine and vinblastine into mouse brain capillary endothelial cells and potently inhibited the basolateral-to-apical flux to digoxin across P-glycoprotein-expressing MDCK cells ($IC_{50}$ < 0.5 μmol/L), indicative of P-glycoprotein inhibition. Inhibition of P-glycoprotein by itraconazole may contribute significantly to the aggravation of vincristine neurotoxicity caused by itraconazole co-administration, and is thought to be

the primary mechanism of the interaction of digoxin and itraconazole, which results in decreased renal clearance of digoxin (Venkatakrishnan, et al., 2000).

The relative contribution of P-glycoprotein inhibition and CYP3A inhibition to the overall effect of ketoconazole or itraconazole on oral clearance and bioavailability of dual substrates of P-glycoprotein and CYP3A such as cyclosporin is not clear, and will require investigation of the magnitude of the interaction in wild type versus *mdr1a* (-/-) knockout mice, or in humans using selective inhibitors of P-glycoprotein and CYP3A. (Venkatakrishnan, et al., 2000).

**Intestinal MDR transport proteins and P-450 enzymes**

Intestinal phase I metabolism and active extrusion of absorbed drug have recently been recognized as major determinants of oral drug bioavailability. Many factors are involved in oral drug delivery, yet, the measured oral bioavailability of a particular drug can be broken down into components that reflect delivery to the intestine (gastric emptying, pH, food), absorption from the lumen (dissolution, lipophilicity, particle size, active uptake), intestinal metabolism (phase I and/or phase II enzymes), active extrusion (drug efflux pumps) and finally first-pass hepatic extraction. The importance of the hepatic first pass metabolism and the ability to quantitate the hepatic extraction has been recognized since the mid-1970s. Here, we will concentrate on intestinal phase I metabolism and intestinal active drug efflux (Rowland and Tozer, 1995).

Both cytochrome P-450 3A4 (CYP3A4), the major phase I drug metabolizing enzyme in humans, and the multidrug efflux pump, MDR or P-glycoprotein (P-gp), are present at high levels in the villus tip enterocytes of the small intestine, the primary site of absorption for orally administered drugs. These proteins are induced or inhibited by many of the same compounds and demonstrate a broad overlap in substrate and inhibitor specificities,

suggesting that they act as a concerted barrier to drug absorption. Clinical studies from our laboratory have demonstrated that the bioavailability of three immunosuppressive agents, cyclosporine, tacrolimus and sirolimus, can be increased by concomitant administration of ketoconazole, a potent CYP3A inhibitor (K, approximately 1 (μM) and an intermediate inhibitor of P-gp (K, approximately 120 μ M) (Benet, et al., 1999).

Conversely, concomitant administration of rifampin, a potent inducers of CYP3A and P-gp, markedly decreased the bioavailability of cyclosporine. A recent clinical study in kidney transplant patients has indicated that variability of intestinal expression of P-gp in humans may be a more important determinant of cyclosporine bioavailability than the variability of intestinal CYP3A. However, the presence of CYP3A is believed to be responsible for the decreased cyclosporine bioavailability. A series of studies in animals have indicated that inhibition of intestinal P-gp has marked effects on the bioavailability of paclitaxel, digoxin and HIV-1 protease inhibitors. We have also recently demonstrated the marked increase in bioavailability of an investigational cysteine protease inhibitor when the drug was dosed concomitantly with ketoconazole to rats. Most recently, we have begun to model the effects of CYP3A and P-gp on intestinal absorption and bioavailability, with the final goal of being able to use *in vitro* measures of drug metabolism by human intestinal CYP3A and bidirectional flux by human MDR transfected cell lines to predict *in vivo* the extent of gut first-pass extraction (Benet, et al., 1999).

1. Intestinal CYP3A4

Enzymes of the CYP3A family are the predominant phase I drug metabolizing species found in humans, accounting for approximately 30% of hepatic CYP and greater than 70% of small intestinal CYP. Moreover, CYP3A

is estimated to metabolize more than half of the drugs that are substrates for the P450 system in humans, although not always as the only, or even the primary, metabolic enzyme (Benet, et al., 1999).

The major congener of the CYP3A family is CYP3A4, the predominant form in adult liver and small intestine. CYP3A4 in these tissues is highly variable with 10-100-fold variations reported for liver and up to 30-fold variations reported for the small intestine (Wacher, et al., 1998). CYP3A levels in the small intestine are generally 10-50% of those found in the liver, however CYP3A concentrations equaling or exceeding the liver levels have been observed in some subjects. CYP3A protein and catalytic activity decrease longitudinally along the small intestine. Although CYP3A4 in the liver and small intestine appears to be the same enzymes, they are not coordinately regulated (Benet, et al., 1999).

2. Intestinal P-gp

P-gp is the product of the multidrug resistance gene *MDR1* in humans and was first characterized as the ATP-dependent transporter responsible for efflux of chemotherapeutic agents from resistant cancer cells. Substrates for P-gp cover a broad range of structures with diverse therapeutic indications. There are no clear structural features defining P-gp substrates, however the molecules tend to be large and amphipathic, containing one or more aromatic rings. P-gp was the first ATP-dependent transporter to be characterized in the liver and represents the best-studied member of the ATP binding cassette family of transporters. P-gp is expressed in a broad spectrum of tissues including the adrenals, bladder, cells of the blood-brain barrier, kidney, liver, lungs, pancreas, rectum, spleen, and significantly for oral drug delivery, the esophagus, stomach, jejunum and colon. In apparent contrast to the observation for CYP3A, P-gp mRNA levels increase longitudinally along the intestine, with lowest levels at the stomach and highest levels in the colon. P-

gp levels also show significant intersubject variability with 2-8-fold variations found in small intestinal biopsies from kidney transplant recipients and healthy volunteers (Benet, et al., 1999).

### 3. Synergistic Actions of Intestinal CYP3A and P-Glycoprotein Play Complementary

#### Roles in Limiting Oral Drug Absorption

The close cellular location of CYP3A4 and P-glycoprotein expression in enterocytes and their similar substrate specificity suggest the importance of these 2 proteins to oral drug delivery. Intestinal CYP3A and P-glycoprotein may act synergistically in the small intestine as a barrier to oral drug bioavailability. The spatial relationship of P-glycoprotein traversing the plasma membrane and CYP3A inside the cell on the endoplasmic reticulum suggests that P-glycoprotein may act to control exposure of substrates to metabolism by CYP3A enzymes. Drug is absorbed by passive processes into the enterocyte where it may be metabolised by CYP3A and also subject to active counter-transport by P-glycoprotein back into the gut lumen. The passive absorption and counter transport may continually cycle a drug between the enterocyte and the gut lumen, thus allowing CYP3A to have repeated access to the drug molecule, possibly at less than saturating concentrations, or leading to nonabsorption, due to the continual counter-transport. The CYP3-mediated metabolism and P-glycoprotein-mediate counter-transport in the enterocyte limit the amount of intact drug that enters into the systemic circulation, and thus decrease drug oral bioavailability (Zhang, 2001).

#### Clinical Studies of Intestinal Drug Metabolism and Efflux Transport Relation to Oral Bioavailabiblity

A recent study by Palkama, et al. (1999). demonstrated that saquinavir markedly increased the oral bioavailability of midazolam. In this double blind,

randomised, 2 phase crossover study, 12 healthy volunteers received oral doses of either saquinavir 1200 mg or placebo 3 times a day for 5 days. On day 3, 6 of the volunteers were given a 7.5 mg oral dose of midazolam and the other 6 received 0.05 mg/kg of intravenous midazolam. On day 5, the volunteers who had received oral midazolam received intravenous midazolam and *vice versa*

Saquinavir increased the oral bioavailability of midazolam from 0.41 to 0.90 and increased the peak drug concentration more than 2-fold. Saquinavir also decreased the clearance of intravenous midazolam by 56% from 0.47 to 0.20 L/h/kg. Midazolam is well absorbed after oral administration (Zhang, 2001).

Midazolam is a well-known CYP3A4 substrate, but not a P-glycoprotein substrate. Midazolam is exclusively metabolised by CYP3A enzymes in humans. Saquinavir is a potent CYP3A inhibitor. This study clearly concludes that saquinavir inhibited both intestinal and hepatic CYP3A-meadiated first-pass extraction of midazolam after oral administration. Similar results were observed by Gorski, et al. (1998) in their drug-drug interaction study of midazolam and clarithromycin, also a CYP3A inhibitor (Zhang, 2001).

Greiner, et al. (1999) recently conducted a clinical study to elucidate the role of intestinal P-glycoprotein in the interaction of digoxin and rifampicin. Rifampicin treatment increased intestinal P-glycoprotein protein level about 3.5-fold, which correlated well with increased AUC of oral digoxin, but not intravenous digoxin. Digoxin is a well-documented P-glycoprotein substrate, but in humans is not subject to metabolism by CYP3A enzymes.