CHAPTER 2

LITERATURE REVIEW

Praziquantel

Praziquantel [2-cyclohexylcarbonyl-(1, 2, 3, 6, 7 11b)-hexahydro-4H-pyrazino (2,1-a) isoquinoline-4-one] (Figure 1), is a heterocyclic pyrazinoline derivatives with a broad-activity against trematodes and schistosomes (Schepmann and Blaschke, 2001).

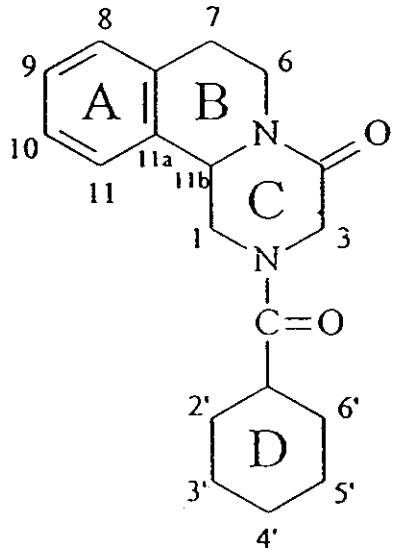

Figure 1 Structural Formula of Praziquantel (Giorgi *et al.*, 2001)

1. Chemical and Physical Properties

Chemical structure : $C_{19}H_{24}N_2O_2$

Molecular weight : 312.41

Solubility (g/100 ml) :

in chloroform	:	56.7
in ethanol	:	9.7
in water	:	0.04

(Budavari, 1989)

2. Pharmocodynamic Properties

2.1 Mechanism of Action

Praziquantel has two prompt actions in susceptible helminths. At the lowest effective concentrations it causes increased muscular activity, followed by contraction and spastic paralysis. Affected worms detach from host tissues, resulting in a rapid shift from the mesenteric veins to the liver. At slightly higher therapeutic concentration, praziquantel causes tegumental damage, which exposes a number of tegumental antigens. Comparisons of stage-specific susceptibility of *S. mansoni* to praziquantel in *vitro* and *in vivo* indicate that the clinical efficacy of this drug correlates better with its tegumental action.

The tegument of anthelmintic seems to be the primary target for praziquantel action, but the molecular mechanism is unknowm. The drug causes an influx of Ca^{2+}across tegument , and the effect is blocked Ca^{2+}-free medium. A number of praziquantel-sensitive sites have been suggested as possible targets (Tracy and Webster, 2001).

2.2 Anthelmintic Activity

The (-) isomer is responsible for most of the drug's anthelmintic activity. The threshold serum concentration of praziquantel for therapeutic effect is about 0.3 μg/ml. In spite of its short half-life, praziquantel is the active agent; its metabolites are inactive.

However, *in vivo* evidence suggests that host antibody to the parasite is also essential to eliminate tissue parasites.

Praziquantel's *in vitro* action on all platyhelminths appears to be increases cell membrane permeability to calcium, resulting in vacuolization, marked contraction, paralysis, dislodgment, and death. Although *F. hepatica* does absorb the drug, no reaction occurs and the infection is not cleared.

In schistosome infections of experimental animals praziquantel is effective against adult worms and immature stages; adult worms are rapidly immobilized and then passively shift to the liver. In addition, when a single high dose of praziquantel is given concurrently with an infecting dose of cercariae, all immature form are killed; thus, praziquantel has a prophylactic effect (Goldsmith, 2001).

2.3 Resistance

Praziquantel resistant *S. mansoni* has been reported from Egypt and Senegal where infection and treatment of mice were used to diagnose resistance (Fallo *at al.*, 1995b; Ismail e*t al.*, 1996). For the detection of praziquantel resistant worms in patients, In Egypt, there is evidence for development of praziquantel resistance in *S. mansoni* (Ismail e*t al.*, 1996), although its true sinificance is not yet clear.

Cross-resistance has not been reported between praziquantel, oxamniquine and metrifonate. Any infection uncured by one drug may still be successfully treated with the appropriate alternative drug (de Silva e*t al.*, 1997).

3. Pharnacokinetic Properties

3.1 Absorption

In man, praziquantel is rapidly absorbed with a bioavailability of about 80% after oral administration and peak plasma levels of unchanged drug of 0.2 to 2.0 μg/ml are achieved in the systemic circulation 1 to 3 hours after administration of therapeutic doses (King and Mahmoud, 1989). Although there is considerable vatiability between individuals, which could be due to difference in metabolism (Sotelo and Jung, 1998).

The absolute bioavailability of praziquantel has not been determined in humans because there is no parenteral formulation. Nevertheless, studies in animals suggest that extensive first pass metabolism occurs with only a small proportion of the active drug reaching the systemic circulation (Sotelo and Jung, 1998).

The relative bioavailabilities of the 3 generic brands compared to the original brand (Bitricide(R) Bayer) ranged from 69.86 to 91.25%. Only one generic brand was considered bioequivalent to the original brand. The observed differences in oral bioavailability was attributed to variable disintegration and dissolution rates due to differences in the manufacturing process and formulation. The mean peak serum concentrations and times to reach the peak ranged from 1.007 to 1.625 mcg/mL and from 1.72 to 2.81 hours, respectively. A decrease in the oral bioavailability of some generic praziquantel brands corresponds to a decrease in dosage and may result in unacceptably high rates of treatment failure (Kaojarern *et al.*, 1989).

The presence of food affects the pharmacokinetic proporties of praziquantel, the AUC_{0-8} increased in the food treatments; the relative bioavailability of the praziquantel was increased by a factor of 2.72 and 3.98 when the drug was administered with the high-lipid and high-carbohydrate diets, respectively. This effect could be related to tablet disintegration, better drug dissolution, or other factors, such as changes in hepatic blood flow or in the metabolism of the drug during the first passage through the liver (Castro *et al.*, 2000). In another study, plasma concentration of praziquantel increased when a high carbohydrate diet was administered, apparently due to inhibition of cytochrome P450, which has a vital role in the mixed function oxidase reactions responsible for praziquantel hydroxylation (Mandour *et al.*, 1990). In addition, fatty meal increased in the C_{max} and the $AUC_{(0-\infty)}$ of praziquantel was 1.7 and 2.6 fold, respectively (Homeida *et al.*, 1994).

The plasma concentrations of praziquantel were fitted to a two-compartment model with first order absorption (Guiniady *et al.*, 1994). On the other hand, Na-Bangchang *et al.* (1993) showed that basic pharmacokinetic parameters of praziquantel were derived by noncompartment model.

3.2 Distribution

Praziquantel is rapidly distributed in body tissues due to its high lipid solubility. Approximately 80 to 85% of the drug is bound to plasma proteins. (Sotelo and Jung, 1998). Praziquantel crosses the blood-brain barrier, reaching cerebrospinal fluid (CSF) concentrations approximately 25% that of plasma levels (de Silva *et al.*, 1997). In addition, small concentrations of drug (< 10% to 20% of plasma levels) have been detected in bile, feces and breast milk (King and Mahmoud, 1989).

3.3 Metabolism and Elimination

In humans, oral praziquantel undergoes extensive first pass hepatic biotransformation into a series of mono- and dihydroxylated products lacking anthelmintic activity. First pass metabolism is dose-dependent with regard to capacity. Leopold *et al.* (1978) observed that doses of 5, 10, 20 and 50 mg/kg produced respective serum concentrations of 0.15, 0.25, 0.80 and 4.22 mg/L. Thus, 2, 4 and 10 times the oral dosage produced 2, 5 and 27 times the serum concentrations. It appears that hepatic first pass metabolism might reach saturation at serum concentrations within the range of 0.64 to 3.2 mmol/L (Sotelo and Jung, 1998). Extensive first-pass metabolism to many hydroxylated and conjugated products limits bioavailability of this drug and results in plasma concentrations of metabolites at least 100 fold higher than that of praziquantel (Tracy and Webster, 2001). Praziquantel is highly metabolized in the liver with a high extraction ratio (Na-Bangchang *et al.*, 1993). Praziquantel is metabolized by the set of cytochrome P450 isozymes induced by phenobarbital (Masimirembwa *et al.*, 1993), producing monohydroxylated derivatives, *trans*-4-hydroxypraziqnantel is the main metabolite in humans (Schepmann and Blaschke, 2001).

The hydroxylated metabolites are also excreted in the urine as conjugates with glucuronic acid (Figure 2) and sulphuric acid (Meier and Blaschke, 2000). The elimination half-life from the serum after a single dose is 1 to 1.5 hours for praziquantel, and for praziquantel metabolites, 4 to 5 hours (Pearson and Guerrant, 1983). And only traces of unchanged drug are recovered in the urine. About 70% of an oral dose of

praziquantel are recovered as metabolites in the urine within 24 hours; most of the remainder is metabolized in the liver and eliminated in the bile (Tracy and Webster, 2001).

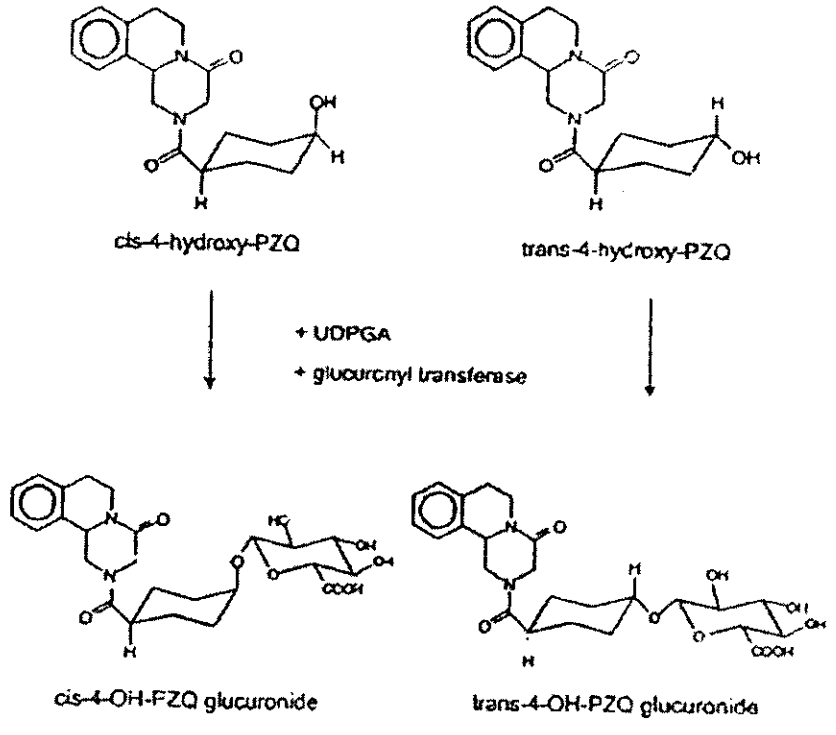

Figure 2 Conjugation of *tran*-and *cis*-4-hydroxypraziquantel with uridine- 5′-diphosphoglucuronide acid (UDPGA) and microsomes containing glucuronyl transferase (Meier and Blaschke, 2000).

4. FDA Labeled Uses

4.1 Clonorchiasis/Opisthorchiasis

4.2 Liver flukes

4.3 Schistosomiasis

5. Therapeutic uses

5.1 Schistosomiasis

Praziquantel is the drug of choice for all schistosomiasis. The dosage, especially for *S. japonicum* infections, is 20 mg/kg at intervals of 4-6 hours for a total of three doses. Other schedules with lower total doses have been effective in some regions, 40 mg/kg in two divided doses for *S.mansoni* and *S. haematobium*. High cure rates (75-95%) are achieved when patients are evaluated at 3-6 months; there is marked reduction in egg counts (92-99%) in those not cured. The drug is effective in adults and children and is generally well tolerated by patients in the hepatosplenic stage of advanced disease. It is not clear, however, whether the drug can be safely or effectively used during the acute stage of the disease (Katayama fever) because release of antigens from dying immature worms may exacerbate symptoms. A related finding is that schistosomes 2-5 weeks old are largely insensitive to praziquantel. Increasing evidence indicates rare *S. mansoni* drug resistance, use of the drug prophylactically has not been established (Goldsmith, 2001).

5.2 Clonorchiasis and Opisthorchiasis

Praziquantel is effective (>95% cure rate) in a single dose 30 or 40 mg/kg for opisthorchiasis (de Silva *et al.*, 1997) or the dosage of 25 mg/kg three times for 1 day for opisthorchiasis and 2 days for clonorchiasis infections results in nearly 100% cure rates (Goldsmith, 2001).

5.3 Paragonimiasis

When treated with 25 mg/kg three times daily for 2 days, cure rates for pulmonary paragonimiasis are 89-100%.

5.4 Taeniasis and Diphyllobothriasis

A single dose of praziquantel 10 mg/kg, results in cure rates of 97-100% for *T. saginata* and *T. solium*. In cysticercosis-endemic areas, it may be safer but equally effective to use 2.5 mg/kg because there was a report that at least one instance of an exacerbation of neurocysticercosis symptoms has occurred following low-dose mass treatment for taeniasis. A sigle dose of 25 mg/kg results in similar cure rates for *D. latum* infections. Within 24-48 hours after treatment, a disintegrating worm is usually passed by normal peristalsis. Pre- and post-treatment purges are not necessary. If the scolex is searched for but not found or is not searched for, cure can be presumed only if regenerated segments have not reappeared 3-5 months after treatment. For *T. solium*, the recommendation continues that an effective purge (e.g., magnesium sulphate, 15-30 g) be given 2 hours after treatment to eliminate all mature segments before eggs can be released from disintegrating segments. Since praziquantel dose not kill the eggs, it is theoretically possible that larvae released from eggs in the large bowel could penetrate the intestinal wall and give rise to cysticercosis. However, as with the use of niclosamide, this hazard is probably minimal (Goldsmith, 2001).

5.5 Neurocysticercosis

Neurocysticercosis should be treated in hospital by a physician with neurologic expertise. The indications, cautions, use of concomitant corticosteroids, and outcome for praziquantel treatment are similar to those for albendazole use. However, in comparable studies, albendazole is the preferred drug. The praziquantel dosage is 50 mg/kg/d in three divided doses for 14 days. If appropriate services are available, blood levels should be monitored. Therapy may result in apparent cure, with clearance of symptoms, changes in cysts by cerebral tomograms (disappearance, reduction in size, or calcification), and clearing of abnormal cerebrospinal fluid findings. In other patients, there may be remarkable improvement, including reduction in cerebral hypertension and amelioration of seizures. A new 1-day therapeutic regimen is being evaluated (three doses of 25 mg/kg at 2 hour intervals) (Goldsmith, 2001).

5.6 *Hymenolepis nana*

Praziquantel is the drug of choice for *H. nana* infections and the first drug to be highly effective. A single dose of 25 mg/kg is taken initially and repeated in 1 weeks (Goldsmith, 2001).

5.7 Other Parasites

Limited trials at a dosage of 25 mg/kg times a day for 1-2 days indicate a high order of effectiveness of praziquantel against fasciolopsiasis, metagonimiasis, and other forms of heterophyiasis. In fascioliasis, however, praziquantel had only a low effectiveness at dosages as high as 25 mg/kg three times daily for 3-7 days (Goldsmith, 2001).

6. Untoward Effects

Mild and transient adverse effects directly attributable to the drug are common. They begin within several hours after ingestion and may persist for hours to 1 day. Most frequent are headache, dizziness, drowsiness, and lassitude; others include nausea, vomiting, abdomen pain, loose stools, pruritus, urticaria, arthralgia, myalgia, and low-grade fever. Minimal to mild transient elevations of liver enzyme have occasionally been reported. Low-grade fever, pruritus and skin rashes (macular and urticarial), sometimes associated with augmented eosinophilia, may also appear several days after starting the medication and are more likely to be due to the release of foreign proteins from dying worms than to a direct action of the drug.

Praziquantel appears to be better tolerated in children than in adults. Adverse effects may be more frequent in heavily infected patients, especially in *S. mansoni* infections. The intensity and frequency of adverse effects also increase with dosage. They are mild and infrequent at dosages of 10 mg/kg given once but occur in up to 50% of patients who receive 25 mg/kg three times in 1 day. Two types of adverse reactions in treatment of neurocysticercosis are (1) those characteristic of praziquantel usage at high dosage (described above) and (2) neurologic reactions or exacerbation of existing ones due to inflammatory reactions around dying parasites. Common findings in up to

90% of patients who do not receive corticosteriods are headache, meningismus, nausea, vomiting, mental changes, and seizures (often accompanied by increased cerebrospinal fluid pleocytosis). These occur during or shortly after completion of therapy, last 48-72 hours, and usually are sufficiently mild that they can be ameliorated with analgesics, antiemetics, diuretics, or anticonvulsants. However, arachnoiditis, hyperthermia, and intracranial hypertention may also occur. Many workers give dexamethasone concurrently in order to decrease the inflammatory reaction; this is controversial, especially with the recent recognition that steroids reduce the plasma level of praziquantel up to 50%. It is not established, however, that the steroidal reduction in plasma levels also reduces the effectiveness of praziquantel (Goldsmith, 2001).

7. Contraindications

Ocular cysticercosis

8. Precautions

8.1 Pregnancy category B; do not nurse on day of treatment and during the subsequent 72 hours

8.2 Children <4 years

8.3 Cerebral cysticercosis (hospitalize patient for duration of therapy)

8.4 Special Precautions

The patient's ability to drive or to operate machinery may be temporarily impaired. Caution should be exercised where there is a possibility of a simultaneous occurrence of both Schistosomiasis and CNS-cysticercosis infection, as cerebral cysticercosis requires hospital-based treatment by a specialist. The safety in pregnancy has not yet been established. A breastfeeding mother can undergo treatment provided her baby is fed otherwise on the day of treatment and during the subsequent 48 hours (Tracy and Webster, 2001).

9. Drug Interaction

9.1 Phenobarbital and 3-Methylcholanthrene

The effect of phenobarbital and 3-methylcholanthrene pretreatment on the pharmacokinetic of praziquantel were studied in Sprague-Dawley rats. The phenobarbital pretreated rats showed a 6-fold decrease in AUC, 6-fold decrease in C_{max} and an 8-fold increase in total Cl compared to the saline treated controls. The 3-methylcholanthrene-pretreated rats and their olive oil treated controls did not show any statistically significant differences in the above parameters. These results suggest that praziquantel is extensively metabolized by phenobarbital-inducible cytochrome P-450 isoforms and not by 3-methylcholanthrene-inducible isoforms. These findings also suggest that the bioavailability of praziquantel could be altered to a significant extent in humans taking drugs that are phenobarbital type induces (Masimirembwa *et al.*, 1993).

9.2 Cimetidine, Ketoconazole and Miconazole (*in vitro*)

The effect of cytochrome P-450 inhibitors on the metabolism of praziquantel was investigated in rats. Cimetidine, ketoconazole and miconazole yielded a 90% inhibition of the metabolism of praziquantel in liver microsome preparations from phenobarbital-pretreated rats at concentrations of 2.0, 0.03 and 0.01 mM, respectively. In rats in vivo ketoconazole and miconazole increased the bioavailability of praziquantel by a factor of 2 and 4, respectively in doses of 25 mg/kg. In phenytoin-pretreated rats ketoconazole increased the bioavailability of praziquantel by a factor of 1.4, whereas miconazole yielded a 5-fold increase of the bioavailability. Cimetidine was an effective inhibitor at a dose of 200 mg/kg. These results suggest that the inhibitors tested may suppress the metabolism of praziquantel in humans and consequently increase the bioavailability and blood levels at doses common in human therapy (Diekmann *et al.*, 1989).

9.3 Cimetidine

A patient had neurocysticercosis complicated by a sizure disorder requiring anticonvulsants; previous praziquantel therapy (50 mg/kg/day) had failed to eradicate the disease. In an attempt to inhibit cytochrome P-450 metabolism of praziquantel, cimetidine (1600 mg/kg/day) was coadministered. Before addition of cimetidine, the maximum concentration praziquantel was 350 ng/ml; concurrent cimetidine administration increased the maximum concentration to 826 ng/ml. The elimination haft-life increased from 1.7 h without cimetidine to 3.3 h with cimetidine and the area under the curve for the 12-h sampling period rose from 754 ng.h/ml to 3050 ng.h/ml. Coadministration of cimetidine raises serum praziquantel levels and may be helpful in patients treated concomitantly with praziquantel and anticonvulsants (Dachman *et al.*, 1994).

Metwally *et al.* (1995) investigated the effect of cimetidine on the bioavailability of the two brand of praziquantel available in normal healthy volunteers. Brand 1 (CAS 55268-74-1) when coadministered with cimetidine showed elevated concentration of the drug 1 hours post treatment. Analysis of the pharmacokinetic parameters revealed insignificant difference comparing brand 1 versus brand 1 plus cimetidine. Significant difference were observed between the elimination rate constant Ke for brand 2 alone (0.017 ± 0.004) versus brand 2 plus cimetidine (0.006 ± 0.001).

Levels of praziquantel in plasma were determined for eight healthy volunteers after the administration of three oral doses of 25 mg/kg given at 2-h intervals, alone and with the simultaneous administration of cimetidine. Levels of praziquantel in plasma remained above 300 ng/ml during a period of 12 h; they increased 100% when cimetidine was jointly administered (Jung *et al.*,1997).

Compared with other regimens, the high level obtained and the longer duration of action seem to be advantageous in prolonging the exposure of the parasites to the drug and support previous clinical experience showing that the treatment of neurocysticercosis with praziquantel can be reduced from 2 weeks to 1 day with the drug still retaining its cysticidal properties (Jung *et al.*,1997).

9.4 Dexamethasone

Simultaneous administration of dexamethasone with praziquantel was shown to reduce plasma levels of praziquantel to approximately 50% as compared with levels when praziquantel was administered alone, but their methodology could not indicate the mechanism of the effect. Dexamethasone should not be added to praziquantel therapy as preventive treatment, but should be reserved for transient therapy of adverse reaction (Vazquez *et al.*, 1987).

9.5 Antiepileptic Drugs

A controlled study demonstrated that carbamazepine reduced the AUC of praziquantel by 90% and the peak plasma level by 92% (Bittencourt *et al.*, 1992). Phenytoin also significantly reduced praziquantel AUC and peak plasma concentration in the same study. Because seizure disorders commonly accompany neurocysticercosis, combined therapy with these agents may frequently be necessary. Cimetidine (an enzyme inhibitor) has been successfully employed in one patient to counteract the enzyme induction caused by phenytoin and phenobarbital (Dachman *et al.*, 1994), however these results have not been confirmed by controlled prospective study.

Plasma praziquantel concentrations were measured in 11 Thai patients with active neurocysticercosis. Praziquantel was given at a daily dose of 45 mg/kg given in three divided doses for 15 consecutive days. After oral administration, the drug was rapidly absorbed from the gastrointestinal tract. There was substantial inter-individual variability in plasma concentrations of praziquantel. The results suggested that the unusual low plasma availability of the drug observed in this group of patient could be a consequence of pharmacokinetic drug interactions of the concomitant therapy with antiepileptic drug (phenytoin or phenobarbital). These drugs would be expected to significantly increase clearance secondary to induction of extensive first-pass metabolism of praziquantel, and relatively low plasma/CSF availability of the drug consequently resulted (Na-Bangchang *et al.*,1995).

9.6 Chloroquine

Because of the possibility of dual infections with malaria and schistosomiasis, the effect of chloroquine on the pharmacokinetics of praziquantel were studied in eight healthy male volunteers. Each participant received an oral dose of praziquantel 40 mg/kg alone and in combination with chloroquine 600 mg. Mean maximum concentrations (C_{max}) of praziquantel alone were 2.13 mcg/mL and decreased to 0.88 mcg/mL in the presence of chloroquine. Area under the concentration-time curve (AUC) of praziquantel also significantly decreased from 11.75 mcg/h/mL to 4.17 mcg/h/mL when given with chloroquine. However, one of the eight volunteers did not have any significant pharmacokinetic alterations, suggesting that a large interindividual variation exists. This has been explained by the wide variation in the metabolism of praziquantel, which undergoes extensive first-pass metabolism. Chloroquine may decrease the curative rate of praziquantel, especially in individuals who appear to metabolize praziquantel more quickly than others. Clinicians should be aware that patients also receiving chloroquine may need increased praziquantel doses (Masimirembwa *et al.*, 1994).

9.7 Albendazole

Study in 10 healthy volunteers demonstrated a mean increase of 50% in mean maximum plasma concentration and AUC of albendazole sulfoxide when praziquantel was administered concurrently. The pharmacokinetics of praziquantel did not change following coadministration with albendazole and, mean T_{max} and mean plasma elimination half life of albendazole remained the same (Prod Info Albenza(R), 1996).

9.8 Grapefruit Juice

After a single oral dose of praziquantel with 250 ml of grapefruit juice, the area under the concentration-time curve and the maximum concentration in plasma of praziquantel (C_{max}) were significantly increased (C_{max} for water treatment,637.71 $\pm$ 128.5 ng/ml; and C_{max} for grapefruit juice treatment, 1,037.65 $\pm$ 305.7 ng/ml, $P < 0.05$). No statistically significant differences were found in the time to maximum concentration of drug in plasma or elimination half-life (Castro *et al.*, 2002).

10. Factors Affecting the Pharmacokinetics of Praziquantel

10.1 Opisthorchiasis Patients

The pharmacokinetic of praziquantel at a single oral dose of 40 mg/kg investigated in 9 patients with early stage infection and 9 patients with moderately advanced stage opisthorchiasis (hepatomegaly). The results indicate the impairment of metabolism of praziquantel in the moderately advanced stage opisthorchiasis. The pharmacokinetics of the drug in these patients during the acute infection was markedly altered when compared with that after recovery and in patients with early stage of the infection. The clearance rate (Cl/f) was significantly reduced and the $t_{1/2}$ and the mean residence time of the drug in the body (MRT) were prolonged. Apparent Vd remain unchanged, suggesting that plasma or tissue protein binding of the drug was not affected by the disease in this condition. In addition, $AUC_{(0-\infty)}$ was sigifcantly greater (Na-Bangchang *et al.*, 1993).

10.2 Schistosomiasis Patients

The pharmacokinetic of praziquantel were investigated in Sudanese patients with hepatosplenic schistosominasis and in healthy volunteers. In patients, higher plasma levels of praziquantel were noted ($P<$ 005 at 8 h) compared to healthy controls; however, due to wide inter-individual variations, there were no significant differences in C_{max}, T_{max}, AUC, Vd or Cl; $t_{1/2}$ was greater ($P<$ 0.05) in patients than controls (Mandour *et at.*, 1990).

10.3 Neurocysticercosis Patients

Praziquantel was measured in plasma in 29 patients with neurocysticercosis. Mean level of praziquantel was 1.64 μg/ml in plasma after dose 50 mg/kg. The drug levels obtained for praziquantel showed ample individual variations that were not related to age, sex, presence of inflammation in the subarachnoid space, or therapeutic effectiveness; such variations seem to be due to individual differences in pharmacokinetics. Praziquantel was effective and the doses currently used of drug seem to be optimal for therapy of neurocysticercosis (Jung *et al.*, 1990).

10.4 Schistosomiasis Patients with and without Liver Cell Failure

The pharmacokinetic and therapeutic efficacy of praziquantel were studied in 40 patients with *S. mansoni* and various degrees of hepatic dysfunction. Every patient was treated with 40 mg/kg of praziquautel as a single oral dose. The pharmacokinietic parameters did not differ significantly between patients with simple active schistosomiasis (group 1) and those with hepatosplenomegaly with liver involvement but without ascites and jaundice (group 2). However, as liver cell dysfunction became more evident (group 3 and 4), pharmacokinetic parameters of praziquantel such as the half-life of elimination, the half-life of absorption, the C_{max}, the T_{max} and the AUC increased proportional to the degree of hepatic insufficiency (Guiniady *et al.*, 1994).

10.5 *Schistosoma japonicum*-Infected Patients with Liver Disease

The influence of liver disease on the pharmacokinetic of praziquantel was studied when administered orally to 30 patients with proven *S. jajonicum* infection whose liver disease was carefully assessed as being severe, moderate or absent. Both the peak plasma concentration of praziquantel and the bioavailability were significantly greater in the two groups of patients with liver disease (P <0.005), as were the concentrations of the two identified metabolites of praziquantel. This result indicates that the side effects and bioavailability of praziquantel are increased in the presence of liver disease (Watt *et al.*, 1988).

10.6 *Schistosoma haematobium*-Infected Subjects

The kinetics of praziqunel was studied in normal and *Schistosoma haematobium*-infected Ghanaian subjects. There was a wide interindividual variation in $t_{1/2}$, C_{max}, T_{max}, $AUC_{(0-\infty\ h)}$ and urinary recovery. No difference were noted between the two groups with regard to T_{max}, $AUC_{(0-8\ h)}$ and $t_{1/2}$. Mean C_{max} was higher in the patients than in the control subjects. The 8-hrs urinary recovery of praziquantel was higher in the subjects with urinary schistosomiasis. The amount of praziquantel excreted unchanged in urine was.0.0052 $\pm$ 0.0027 % of the dose for the control subjects and 0.0054 $\pm$ 0.0027 % for the patients (Ofori-Adjei *et al.*,1988).

10.7 Renal Failure

Investigators reported that the pharmacokinetics of praziquantel were unchanged in a patient with underlying kidney disease due to *S. haematobium* treated with praziquantel (Pehrson *et al.*, 1983). Renal elimination of unmetabolized praziquantel was changed only slightly and dosing adjustments did not appear necessary.

10.8 Liver Insufficiency

Liver blood flow is reduced in advanced stages of schistosomiasis. Antipyrine clearance may remain unchanged or be slightly reduced. Chronic liver disease secondary to schistosomiasis is not true cirrhosis and hepatocyte dysfunction is reported to be minimal until the very last stages of the disease. Dosage recommendations for patients with liver disease are controversial (Edwards and Breckenridge, 1988). Some clinicians recommend a dosage reduction in patients with major hepatic insufficiency or portocaval shunts since disproportionately high concentrations of unmetabolized praziquantel could be expected to reach the systemic circulation (Leopold *et al.*, 1978; Patzschke *et al.*, 1979).

However, others have found no significant differences in concentrations of praziquantel metabolites in the serum and urine of patients with secondary hepatomegaly and have concluded that dosage adjustment is not necessary in such patients (Edwards and Breckenridge, 1988). The incidence of drug-related adverse effects with normal therapeutic doses of praziquantel was not increased in patients with hepatosplenic complications due to advanced disease (Wegner, 1984).

Azole Antimycotics

Azole antifungals include two broad classes, imidazoles and triazoles. Both classes share the same antifungal spectrum and mechanism of action (Chambers, 2001). The azole antifugals are less toxic than amphotericin B and effective in many different fungi (Lyman and Walsh, 1992). The systemic triazoles are more slowly metabolized and have less effect on human sterol synthesis than do the imidazoles. Because of these advantages, new congeners under development are mostly triazole, not imidazole. Of the drugs now on the market in the United States, clotrimazole, miconazole, ketoconazole, econazole, butoconazole, oxiconazole, and sulconazole are imidazoles; terconazole, itraconazole, and fluconazole are triazoles (Chambers, 2001).

1. Pharmacodynamic Properties

1.1 Mechanism of Action

At concentration achieved during systemic use, the major effect of azole antifungal agents on fungi is inhibition of sterol 14-α-demethylase, a microsomal cytochrome P450-dependent enzyme system. Imidazoles and triazoles thus impair the biosynthesis of ergosterol for cytoplasmic membrane and lead to the accumulation of 14-α-methylsterols. These methylsterols may disrupt the close packing of acyl chains of phospholipids, impairing the function of certain membrane-bound enzyme systems such as ATPase and enzymes of the electron transport system and thus inhibiting growth of the fungi (Chambers, 2001). At high concentrations, the imidazoles cause K^+ and other components to leak from the fungal cell. Inhibition of plasma membrane ATPase is a secondary action that may contribute to or help account for the K^+ loss. Because imidazoles inhibit fungal respiration under aerobic conditions, an alternative mechanism may be blockade of respiratory-chain electron transport (Wispelwey and Neu, 1998).

1.2 Antifungal Activity

Susceptibility testing with azole antifungals has not been useful in predicting which fungal species will respond to therapy. Although individual drugs have their own useful spectrum, azoles as a group have clinically useful activity against *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. neoformans*, *B. dermatitidis*, *H. capsulatum*, *C. immitis*, *P. brasiliensis*, and ringworm fungi (dermatophytes). *Aspergillus* spp. and *S. schenckii* are intermediate in susceptibility. *C. krusei* and the agents of mucormycosis appear to be resistant. These drugs do not appear to have any useful antibacterial or antiparasitic activity, with the possible exception of antiprotozoal effects against *Leishmania major* (Chambers, 2001).

1.3 Resistance

Azole resistance has emerged gradually during prolonged azole therapy and has caused clinical failure in patients with far-advanced HIV infection and oropharyngeal or esophageal candidiasis. The primary mechanism of resistance in *C. albicans* is accumulations of mutation in ERG11, the gene coding for the C14-α-sterol demethylase. These mutations appear to protect heme in the enzyme pocket from binding to azole but allow access of the natural substrate for the enzyme, lanosterol. Cross resistance is conferred to all azoles. Increased azole efflux by both ATP-binding cassette (ABC) and major facilitator superfamily (MFS) transporters can add to fluconazole resistance in *C. albicans* and *C. glabrata*. Increased production of C14-α-sterol demethylase is another potential cause of resistance (Chambers, 2001).

Ketoconazole

Ketoconazole is *cis*-1-acetyl-4-[*p*-[[2-(2, 4-dichlorophenyl)-2-imidazol –1- ylmethyl) 1,3-dioxolan-4-yl] methoxy] phenyl] piperazine (Córdoba-Díaz *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 antifungal 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 2)

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)

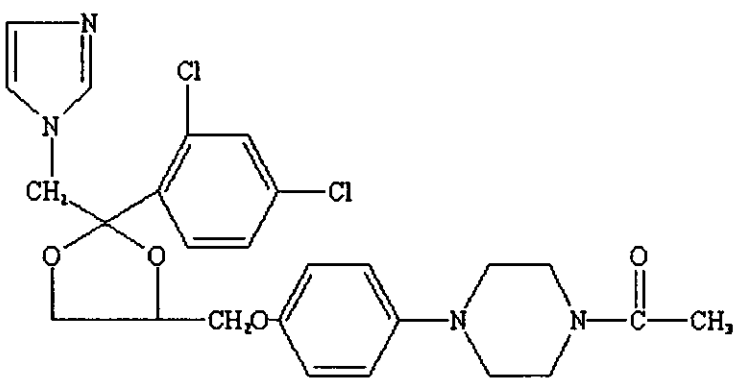

Figure 3 Structural Formula of Ketoconazole

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 for the dissolution of ketoconazole, bioavailability is markedly depressed in patients taking H_2–histamine receptor blocking agents such as antacid, cimetidine or proton pump inhibitors, thus should take 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 Tyle, 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 *el 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 *N*-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 faeces and 13% in urine). Of the faecal 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 ketoconazole (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

Ketoconazole 300 milligrams 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 al.*, 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 al.*, 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.*, 1984).

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; Balbi *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 posttreatment 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 was 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 have 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 are 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 pateints 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-17β. 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 burns, 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). Coadministration 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 (Olkkola *et al.*, 1994). Significant increases in sedative effects were indicated by psychomotor tests and subjective reporting of drowsiness with the combination. 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. Coadministration 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, amlodipine, isradipine, and felodipine (Guengerich *et al.*, 1991; Josefsson *et al.*, 1996). 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 U-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 coadministration with fluvoxamine (which inhibits CYP1A2 and to some extent CYP2C19) had no significant effect on the mean apparent oral clearance of quinine. Coadministration of ketoconazole also decreased the mean AUC of 3-hydroxyquinine, whereas coadministration 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 contries for treatment of depression. It is metabolized by CYP3A4.

Eleven healthy volunteers received 4 mg reboxetine orally on the 2nd day of a 5 days regimen of 200 mg ketoconazole once daily in a crossover design. Ketoconazole increased R, R(-) – reboxetine and S, S (+)-reboxetine (more active reboxetine enantiomers) mean AUC by 58% and 43%, respectively ($P < 0.02$). Oral clearance of both enantiomers were 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) were 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 coadministered (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 Antacids

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; Carlson *et al.*, 1983).

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 which 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).

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.

Itraconazole

Itraconazole{4-[4-[4-[4-[[2-(2,4-Dichlorophenyl)-2-(1*H*-1,2,4-triazol-l-ylmethyl)1,3-dioxolam-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2-(1-methylpropyl)-3*H*-1,2,4 triazol-3-one} was first synthesized in 1980. The structural formula of itraconazole is closely related to ketoconazole, as shown figure 4, Itraconazole is a water insoluble, lipophilic triazole analogue. When given orally, it was 5 to 100 times more active than ketoconazole (Heeres *et al.*, 1984). In addition, unlike ketoconazole, it was active in aspergillosis, meningeal cryptococcosis and sporotrichosis and more desirable pharmacokinetic profile and less toxicity (Warnock, 1989).

1. **Chemical and Physical Properties**

Chemical structure : $C_{35}H_{38}Cl_2N_8O_4$ (Figure 3)

Molecular weight : 705.6

pKa : 3-4

Solubility

in dimethyl sulfoxide : > 1 in 100 (w/v)

in alcohol : 1 in 1,000 (w/v)

in water : < 1 in 1,000,000 (w/v)

Octanol/water partition coefficient : 46,000 (pH 8.1)

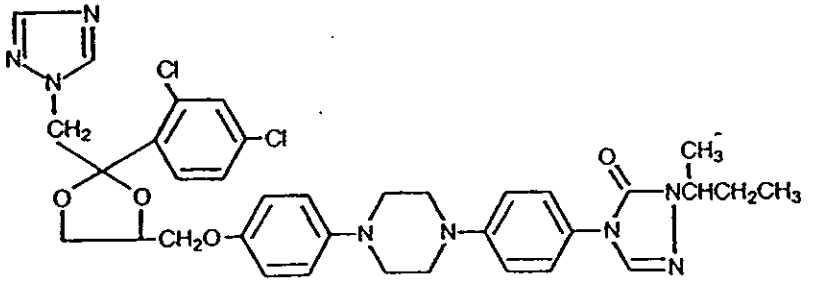

Figure 4 Structural formula of Itraconazole

2.Pharmacokinetics Properties

Itraconazole is highly lipophilic and virtually insoluble in water. It is an extremely weak base, ionized only at low pH, such as that in gastric fluids. Basic pharmacokinetic of itraconazole in humans are presented below.

2.1 Absorption

Following oral administration, itraconazole is rapidly and extensively absorbed with absolute systemic bioavialability of 55%. Systemic bioavailability of itraconazole capsules is maximized when taken with food (Prod Info Sporanox(R) capsules, 2001).

In a randomised crossover study, healthy volunteers were given itraconazole 100 mg either as an intravenous infusion or an oral aqueous acidified solution in 5% hydroxypropy 1-β-cyclodextrin. The absolute bioavailability of oral itraconazole was 55%. For more ease in use, the drug formulation selected for therapy was a capsule, bioequivalent to the solution. Peak drug concentrations (C_{max}) in plasma were attained 3 to 4 hours after oral intake. In a recent study comparing itraconazole capsules with a new cyclodextrin solution in HIV positive patients, Cartledge *et al.* (1997) showed that serum C_{max} values were 59% higher ($p < 0.005$) in patients receiving solution than in those receiving capsules (Poirier and Chemo, 1998). Itraconazole is only ionized at low pH, such as in the gastric milieu. The plasma concentration time curves of itraconazole in healthy volunteers were a wide interindividual and steady-state pharmacokinetics of 200 mg itraconazole once daily and twice daily in 6 healthy volunteers. The C_{max} and AUC observed for the once daily dosage was 1.1 mg/l and 15.4 mg/l hr, respectively and for the twice a day dosage 2.0 mg/l and 39.3 mg/l.hr. Thus, dosage increase between 100, 200 and 400 mg daily produced non-linear increases in the AUC, suggesting that possibility of saturable metabolic processes (Hardin *et al.*, 1988). Mean peak concentrations of 0.02 mg/l are attained when a 100 mg dose is administered during fasting, while peak concentrations of 0.18 mg/l are attained when the drug is administered after feeding (Wishart, 1987).

2.2 Distribution

99.8% of the absorbed drug binds to human plasma proteins, primarily albumin, so that the concentrations of unbound itraconazole in body fluids (cerebrospinal fluid (CSF), eye fluid and saliva) are low in relation to plasma concentrations. The relationship between protein binding and antifungal activity is not well understood. For example, both itraconazole and amphotericin B are highly protein bound, yet they exhibit a potent antifungal effect. Itraconazole has a high affinity for tissues achieving concentrations 2 to 10 times higher than those in plasma. The apparent volume of distribution is about 11 L/kg. The drug maintains high concentrations in vaginal tissue and nails. Its concentration in the vaginal mucosa

remains elevated for at least 2 days after it is no longer detectable in the blood. In addition, it is accumulated in the horny layer of the nails for more than 10 days. (Poirier and Cheymol, 1998).

2.3 Metabolism and elimination

Itraconazole is extensively metabolised by the liver, unmetabolised itraconazole is not detected in the urine and only 3 to 18% of the dose given is detected as the parent drug in the faeces. One of the many metabolites formed is hydroxy-itraconazole, which has been particularly well studied because its antifungal activity in vitro is similar to that of itraconazole. In addition, the plasma concentrations of the metabolite are higher than those of the parent drug : after a single oral dose the ratio of plasma concentration-time curves (AUC) was 2.3 and the mean steady-state plasma concentration of the metabolite was almost double that of itraconazole. No information is available about the binding of hydroxy-itraconazole to plasma proteins.

The terminal elimination half-life ($t_{1/2}$) of a single oral dose was 24 $\pm$ 9 hours (mean $\pm$ SD) for itraconazole and about 14 hours for its hydroxylated metabolite. There is an apparent contradiction between these $t_{1/2}$ values, but they are approximate. A multiple dose study (itraconazole 200 mg twice daily) showed Michelis-Menten kinetics when the plasma concentrations were measured for up-to 15 days after the last dose.

Mean total blood clearance, measured in 6 healthy volunteers who were given itraconazole 100 mg as an intravenous infusion, was 39.6 $\pm$ 10.2 L/h (mean $\pm$ SD). Assuming that most clearance occurs in the liver (negligible amounts recovered in urine sample), the hepatic extraction ratio should be 0.5, so that the maximal oral bioavailability should be 50%, close to the reported value of 55 $\pm$ 15%.

Some of the pharmacokinetic data obtained in healthy volunteers are of particular importance to therapy. First, repeated doses produced a steady state within 2 weeks of the start of treatment. Which is not consistent with the $t_{1/2}$ calculated after a single dose. Secondly, itraconazole had dose dependent kinetics after single or repeated oral doses of 100 to 400 mg; however, plasma concentrations of the parent drug were higher than was expected had the response been strictly dose-proportional.

This suggests saturation of the metabolism. Comparison of AUC values after 2 to 3 weeks long term treatment with those achieved after a single dose showed that the AUC was 4 to 5 times greater, and the $t_{1/2}$ was slightly longer (up to 30 hours) after prolonged administration. This is typical of nonlinear pharmacokinetic behaviour. The third major feature is that there are interindividual variations with regard to changes in AUC of itraconazole plasma concentration over time, after both single and multiple 200 mg doses of the drug [coefficient of variation (CV) of AUC = 47 and 44%, respectively] (Poirier and Cheymol, 1998).

3. FDA Labeled Uses
   - 3.1 Allergic bronchopulmonary aspergillosis
   - 3.2 Aspergillosis
   - 3.3 Aspergillus meningitis
   - 3.4 Blastomycosis
   - 3.5 Candidiasis-oropharyngeal/esophageal
   - 3.6 Cutaneous aspergillosis
   - 3.7 Empiric treatment
   - 3.8 Febrile neutropenia
   - 3.9 Histoplasmosis
   - 3.10 Mucocutaneous candidiasis
   - 3.11 Onychomycosis
   - 3.12 Sino-orbital aspergillosis
   - 3.13 *Tinea unguium*

4. Therapeutic uses

4.1 Blastomycosis, histoplasmosis, aspergillosis 200mg PO QD, (maximum 200 mg BID)

4.2 Esophageal candidiasis 100-200mg solution swish/swallow QD x 2 weeks following symptom resolution

4.3 **Blastomycosis, histoplasmosis, aspergillosis life-threatening,** 200mg IV BID x 4 doses then 200 mg IV QD

4.4 **Oropharyngeal candidiasis** 100 mg solution swish/swallow QD x 1-2 weeks

4.5 **Blastomycosis, histoplasmosis, aspergillosis life-threatening,** 200 mg PO TID x 3 days then 200 mg PO QD

4.6 **Onychomycosis toenail,** 200 mg PO QD x 12 weeks

4.7 **Onychomycosis fingernail,** 200 mg PO BID x 1 week, off drug x 3 weeks, repeat 200 mg BID x 1 week

A. Aspergillosis

FDA labeled indication. Oral capsules and intravenous itraconazole are indicated for the treatment of extrapulmonary and pulmonary Aspergillosis in immunocompromised and non-immunocompromised patients, when intolerant or refractory to amphotericin B.

B. Blastomycosis

Oral itraconazole in usual doses of 200 to as high as 600 milligrams daily, alone or in combination with other antifungal agents (ie, flucytosine, amphotericin B), has been effective in the treatment of pulmonary and extrapulmonary blastomycosis. Itraconazole 200 to 400 milligrams daily (in twice daily dosing) was successful in treating pulmonary and extrapulmonary blastomycosis in 90% of patients

C. *C. albicans* keratitis

Two patients with *C. albicans* keratitis responded to oral itraconazole 400 milligrams once and twice daily, respectively. Topical amphotericin was concomitantly administered in the second case (Klotz and Bartholomew, 1996).

D. Candidiasis-vaginal

Oral itraconazole 400 milligrams for 1 day (in 2 divided doses) have been similarly as effective as 200 milligrams once daily for 3 days in acute vaginal candidiasis, each producing mycological cure in approximately 82% of patients (Cauwenbergh, 1987). However, in a further study, clinical and mycological cure was observed in only 65%, 55%, and 75% of patients with acute vaginal candidiasis

following itraconazole 200 milligrams daily for 2 days, 200 milligrams twice daily for 1 day, and 200 milligrams daily for 3 days, respectively (Sanz and Hernanz, 1987). While oral itraconazole has exhibited sporadic efficacy in vaginal candidiasis, relief of local symptoms may not be realized within the first 24-48 hours, and severe vulvovaginal irritation may require adjunct topical treatment during that time period (Sobel, 1994).

E. Chromoblastomycosis

Oral itraconazole in divided doses of 200 to 400 milligrams daily was effective or partially effective in 19 Brazilian patients with chromoblastomycosis. The duration of infection before treatment with itraconazole ranged from 4 years to 29 years. Many of these patients had failed other treatments such as surgical excision or amphotericin B. Eight of 19 obtained clinical and mycological cure. The remaining 11 patients showed improvement (Queiroz-Telles *et al.*, 1992).

F. Coccidioidomycosis

Itraconazole was safe and effective in the treatment of 16 patients with coccidioidomycosis (Diaz *et al.*, 1991). Patients received oral itraconazole 400 mg/day for a 1-year period. Fifteen of the 16 patients had negative cultures during the third month of therapy. Itraconazole was well tolerated; none of the patients had to discontinue therapy due to adverse effects.

G. Cryptococcosis

Following 3 months of oral itraconazole 200 milligrams twice daily therapy, complete resolution of eye lesions occurred in the patient with cryptococcal endophthalmitis. The patient had severe disseminated cryptococcosis and AIDS (Denning *et al.*, 1991).

H. Dermatomycosis

Itraconazole treated patients showed a significantly better overall response compared to placebo in patients with *T. versicolor* (p less than 0.01). Thirty-six patients received oral traconazole 200 mg orally for 7 days or placebo. At 5 weeks 67% of the traconazole-treated group were symptom-free compared to 12% of those patients receiving placebo (Hickman, 1996).

I. Febrile neutropenia

Itraconazole (intravenous followed by oral solution) provided comparable efficacy to amphotericin B for the empiric therapy in febrile neutropenic patients. Success was defined as resolution of fever and neutropenia within 28 days of treatment; absence of emergent fungal infections; no discontinuation of therapy due to toxicity or lack of efficacy; and treatment for 3 or more days. Based on intent- to-treat analysis the success rate was similar between itraconazole and amphotericin B (47% and 38%, respectively) (Prod Info Sporanox(R), injection, 2001).

J. Histoplasmosis

Oral itraconazole in usual doses of 200 to as high as 600 milligrams daily, alone or in combination with other antifungal agents (ie, flucytosine, amphotericin B), has been effective in the treatment of histoplasmosis, including chronic cavitary pulmonary disease and disseminated, non-meningeal histoplasmosis (Sharkey *et al.*, 1991).

K. Leishmaniasis

Higher doses of oral itraconazole (7 milligrams/kilogram daily for 3 weeks) compared to placebo was not effective as a single agent for 131 patients with cutaneous leishmaniasis (Momeni *et al.*, 1996). In this double-blind placebo-controlled trial, clinical healing at 1 month occurred in 59% in the treatment group and 44% with the placebo group. Adverse effects were similar for both groups.

L. Mycetoma

Itraconazole promoted healing of lesions associated with mycetoma caused by *Acremonium falciforme* in a 72-year-old man. The eschar-like lesions, located on the right temporal area, were large, black, erythematous, and unresponsive to prior antibiotic therapy. Oral itraconazole was given at a dose of 200 milligrams/day for 70 days, with complete eradication of all lesions and residual scars (Lee *et al.*, 1995).

M. Onychomycosis

Therapeutic itraconazole concentrations in the nail plates of fingernails and toenails has been reported to occur for up to 6 months after treatment in 39 patients with

onychomycosis. Patients received oral itraconazole 100 or 200 mg daily for 3 months. At 6 months after discontinuation of therapy, 79% and 26% had toenail cures with the 200 and 100 mg/day dosage, respectively (Willemsen *et al.*, 1992).

N. Paracoccidioidomycosis

Paracoccidioidomycosis was treated with oral itraconazole in 47 patients, achieving mycologic cure in 87% during the first month of treatment. Clinical signs were markedly improved, while dyspnea, productive cough, and adrenal insufficiency continued in some patients. Radiologic evidence of lesions was noted in 67% of patients prior to therapy and in 13% after therapy. There were no relapses among 15 patients evaluated at 12-month follow-up (Naranjo *et al.*, 1990).

O. Sporotrichosis

Twenty-five of thirty patients with either systemic or cutaneous sporotrichosis were treated successfully with oral itraconazole. Of these patients, 11 did not respond to previous agents such as amphotericin B, SSKI, ketoconazole, and fluconazole (Sharkey-Mathis, 1993). Cutaneous sporotrichosis in 18 patients was successfully treated with cumulative itraconazole doses ranging from 3.1 to 14.8 grams after a mean therapy duration of 44 days. Within a 26-month follow-up, there were no occurrences of relapse or serious adverse effects (Conti-Diaz, 1992).

5. Untoward Effects.

For 834 clinical trial patients receiving 2 to 4 cycles of 1 week therapy, the most frequently reported adverse events during the treatment and follow-up period were: abdominal pain (1.9%), nausea (1.6%) and headache (1.3%). The following adverse experiences have been reported at an incidence greater than 0.05% and less than 1% during short-term therapy with itraconazole: dyspepsia/epigastric pain/upset stomach; abdominal pain/discomfort; vomiting; pyrosis; diarrhea; gastritis; flatulence/meteorism; constipation; decreased appetite; other gastric complaints; dizziness/faintness; sleepiness/somnolence; vertigo; pruritus; rash; pain; fatigue; fever; edema; allergic reaction. Allergic reactions (such as pruritus, rash, urticaria and angioedema) and

reversible increases in hepatic enzymes, and menstrual disorder have been reported from postmarketing experience. Isolated cases of peripheral neuropathy and of Stevens-Johnson syndrome have been reported; a causality for the latter has not been established. If neuropathy occurs that may be attributable to itraconazole, the treatment should be discontinued (Prod Info Sporanox(R), 1997; Prod Info Sporanox(R), 1999; Prod Info Sporanox(R), 2001).

The following adverse experiences have been reported at an incidence of greater than 0.5% but less than 1% of patients during long-term therapy with itraconazole: vomiting; dyspepsia/epigastralgia; diarrhea; abdominal pain; dizziness; bronchitis/bronchospasm; coughing; dyspnea; rhinitis; sinusitis; increase in liver enzymes; abnormal liver function tests; jaundice; hepatitis; cirrhosis; hepatocellular damage; abnormal hepatic function; pain; chest pain; hypertension; fatigue; fever; hypokalemia (Prod Info Sporanox(R), 1997; Prod Info Sporanox(R), 1999; Prod Info Sporanox(R), 2001).

Postmarketing: Especially in patients receiving prolonged (approx. 1 month) treatment, most of whom had major underlying pathology and multiple concomitant medications, cases of hypokalemia, edema, hepatitis and hair loss have been observed (Prod Info Sporanox(R), 1997; Prod Info Sporanox(R), 1999; Prod Info Sporanox(R), 2001).

Itraconazole is well tolerated at 200 mg daily. Most of the adverse reaction reported are transient, and include gastrointestinal disturbances, dizziness, headache, depressed libido with normal testosterone levels and leukopenia (Graybill *et al.*,1990). The drug has a low incidence of hepatotoxicity, with less than 3% of patients experiencing transient elevations in liver function test (Cauwenbergh *et al.*, 1987). It has no effect on testicular or adrenal steroidogenesis (Van *et al.*, 1987). In a series of 189 patients receiving 50 to 400 mg/day, nausea and vomiting were recorded in 10%, hypertriglyceridemia in 9%, hypokalemia in 6%, increased serum aminotransferase in 5%, rash in 2%, and at least one side effects in 39% (Tucker *et al.*, 1990). Profound hypokalemia has been seen in patients receiving 600 mg or more daily (Sharkey *et al.*,

1991). Doses of 300 mg twice daily have led to other side effects, including adrenal insufficiency, lower limb edema, hypertension and in one patient rhabdomyosis (Sharkey *et al.*, 1991). The evidence of side effects increase with duration of treatment.

6. Contraindication

A. Itraconazole coadministration with astemizole, cisapride, dofetilide, midazolam, pimozide, quinidine,lovastatin, simvastatin, or triazolam.

B. The treatment of onychomycosis to patients with evidence of ventricular dysfunction (congestive heart failure (CHF) or history of CHF)

C. Previous hypersensitivity to itraconazole.

D. The treatment of onychomycosis to pregnant patients or to women contemplating pregnancy.

7. PRECUTIONS

7.1 **Ventricular dysfunction** (congestive heart failure (CHF) or history of CHF)-benefit must outweigh the risk.

7.2 **Ischemic and valvular disease** (risk for CHF)

7.3 **Significant pulmonary disease** (chronic obstructive pulmonary disease) (risk for CHF)

7.4 **Renal failure** (risk for CHF)

7.5 **Other edematous disorders** (risk for CHF)

7.6 **Previous hypersensitivity to other azole derivatives**

7.7 **Hepatic dysfunction** (potential hepatotoxicity, idiosyncratic hepatitis; potential need for dose adjustments)

7.8 **Administer itraconazole capsules with food to assure optimal bioavailability**

7.9 **Itraconazole capsules and solution should not be used interchangeably**

7.10 **Patients with renal impairment** (creatinine clearance less than 30 mL/min) and intravenous itraconazole administration.

8. Drug Interaction

8.1 Oral Anticoagulants

Itraconazole is known to enhance the anticoagulant effects of coumarin-like drugs (Prod Info Sporanox(R), 2000). Because the metabolic and pharmacologic properties of acenocoumarol are similar to those of warfarin, all warfarin-associated drug interactions should also be considered possible with acenocoumarol.

8.2 Benzodiazepines

In two controlled studies, the coadministration of triazolam with itraconazole resulted in significantly increased triazolam maximum concentration (C_{max}), area under the concentration-time curve (AUC), and half-life (Neuvonen *et al.*, 1996; Varhe *et al.*, 1994). Other studies have shown similar interactions between triazolam and other azole antifungals such as fluconazole and ketoconazole (Varhe *et al.*, 1996; Greenblatt *et al.*, 1995). Concomitant use of itraconazole and triazolam is contraindicated (Prod Info Sporanox(R), 2000).

Concomitant use of itraconazole 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). Studies have shown itraconazole to be a potent inhibitor of cytochrome P450 3A (CYP3A) enzymes, an enzyme subfamily thought to be important in alprazolam metabolism. Because the initial step in alprazolam metabolism is hydroxylation catalyzed by CYP3A, itraconazole may have a profound effect on the clearance of alprazolam. Concomitant administration of these two agents is contraindicated (Prod Info Xanax(R), 1997).

Concurrent use of oral midazolam and itraconazole is contraindicated, and the concurrent use of intravenous midazolam and itraconazole should be avoided if possible (Prod Info Sporanox(R), 2000). Substantial increases in oral midazolam peak plasma concentration (240%), AUC (980%), and half-life (180%) have been demonstrated to occur with concurrent oral itraconazole compared to placebo in healthy volunteers (Olkkola *et al.*, 1994). In addition, itraconazole and fluconazole have been

shown to reduce the clearance of intravenous midazolam (Olkkola *et al.*, 1996). Significant increases in sedative effects were indicated by psychomotor tests and subjective reporting of drowsiness with the combination. Itraconazole is a known inhibitor of the cytochrome P450 3A4 enzyme system, and midazolam metabolism is mediated through CYP450 3A4. Coadministration of these two drugs may result in prolonged sedation due to reduced midazolam plasma clearance (Prod Info Versed(R), 1997).

8.3 Oral Hypoglycemic Drugs

Concomitant therapy with azole antifungal agents and oral hypoglycemic drugs has been reported to result in severe hypoglycemia; blood glucose determinations should be carefully monitored when these two classes of drugs are coadministered (Prod Info Sporanox(R), 2000).

8.4 Alfentanil

In vitro data indicate that alfentanil is metabolized by cytochrome P450 3A4 (CYP3A4) enzymes. Coadministration with itraconazole, a CYP3A4 inhibitor, may lead to increased alfentanil plasma concentrations and an increased risk of alfentanil toxicity (Prod Info Sporanox(R), 2000).

8.5 Calcium channel blocking agents

Itraconazole inhibits hepatic cytochrome isoenzyme CYP3A4 (Gibaldi, 1992; Prod Info Sporanox(R), 2000), an enzyme involved in the metabolism of some dihydropyridine calcium channel antagonists, including nifedipine, isradipine, nicardipine, amlodipine, and felodipine (Guengerich *et al.*, 1991; Josefsson *et al.*, 1996). 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).

8.6 Antacids

Itraconazole absorption is improved with food and in the presence of normal gastric acidity. Reduced itraconazole plasma concentrations have been reported with concurrent H_2 antagonist and antacid use (Patterson *et al.*, 1996). Antacids should be administered at least one hour before or two hours after itraconazole capsules (Prod Info Sporanox(R), 1999).

8.7 antihistamine drugs

Concomitant use of itraconazole and astemizole has resulted in elevations in astemizole serum concentrations (Prod Info Sporanox(R), 2000). Elevated astemizole serum concentrations have been associated with prolongation of the corrected QT interval and severe, potentially fatal, ventricular arrhythmias (Anon, 1993; Hoppu *et al.*, 1991; Snook *et al.*, 1988; Lefebvre *et al.*, 1997). Concomitant use of astemizole and itraconazole is contraindicated (Prod Info Hismanal(R), 1998; Prod Info Sporanox(R), 2000).

Coadministration of itraconazole and terfenadine has resulted in elevated terfenadine plasma concentrations, which have led to rare occurrences of life-threatening cardiac arrhythmias and one death (Prod Info Sporanox(R), 1997; Pohjola-Sintonen *et al.*, 1993; Crane and Shih, 1993). A controlled study of six healthy volunteers observed increased unmetabolized terfenadine levels and QT interval prolongation in all six subjects during coadministration of itraconazole with terfenadine (Honig *et al.*, 1993). Therefore, the concurrent use of itraconazole and terfenadine is contraindicated (Prod Info Sporanox(R), 1997).

8.8 HMG-CoA reductase

The risk of myopathy is increased when azole antifungals are administered concurrently with HMG-CoA reductase inhibitors such as atorvastatin, simvastatin, or lovastatin. Caution is warranted if concurrent administration is deemed necessary (Prod Info Lipitor(R), 2000). Itraconazole has been shown to inhibit the metabolism of atorvastatin, resulting in an increase in the half-life of atorvastatin by about 3-fold

(Kantola *et al.*, 1998), in the area under the concentration-time curve (AUC) of 150% and in the maximum serum concentration (C_{max}) of 38% (Mazzu *et al.*, 2000).

8.9 H_2 antagonists

H_2 antagonists have been reported to reduce absorption of imidazole antifungal drugs, presumably as a result of altered gastric pH (Van Der Meer *et al.*, 1980). Itraconazole absorption is improved with food and in the presence of normal gastric acidity (Patterson *et al.*, 1996). Because reduced itraconazole plasma concentrations have been reported with concurrent H_2 antagonist use, itraconazole should be administered with a cola beverage in patients receiving an H_2 antagonist (Prod Info Sporanox(R), 1999).

8.10 Tranquilizers

Itraconazole may inhibit the metabolism of pimozide, resulting in increased serum concentrations of this agent. Elevated serum levels of pimozide have been associated with adverse cardiovascular effects including QT interval prolongation, cardiac arrhythmia, and sudden death. The concurrent use of itraconazole and pimozide is contraindicated (Prod Info Orap(R), 1999; Prod Info Sporanox(R), 2000).

8.11 Buspirone

Buspirone is non-benzodiazepine anxiolytic agent that acts as a partial agonist at serotonin receptor of $5HT_{1A}$ type. The oral bioavailability of buspirone is very low as a result of first-pass metabolism. Pretreatment of itraconazole 200 mg/day for 4 days increased the mean area under the plasma concentration-time curve from time zero to infinity ($AUC_{(0-\infty)}$) of buspirone about 19-fold ($P < 0.05$) compared with placebo. The mean C_{max} of buspirone was increased about 13-fold ($P < 0.01$) by itraconazole. These interactions were evident in each subject, although a striking interindividual variability in the extent of the elimination half-life of buspirone was not prolonged by itraconazole. The greatly elevated plasma buspirone concentrations resulted in increased ($P < 0.05$) pharmacodynamic effects and in side effects of buspirone. The interaction caused by inhibiting its CYP3A4 mediated first-pass metabolism (Kivisto *et al.*,1997).

8.12 Cisapride

Concomitant administration of cisapride and ketoconazole has resulted in decreased cisapride metabolism, increased cisapride plasma concentrations, and prolonged QT interval. In vitro data suggest that itraconazole also significantly inhibits the metabolism of cisapride (Shulman, 1996). Concomitant use of cisapride and itraconazole is contraindicated (Prod Info Sporanox(R), 2000; Prod Info Propulsid(R), 2000).

8.13 Quinidine

Itraconazole is known to inhibit cytochrome P450 3A4, an isoenzyme involved in quinidine metabolism. Concomitant therapy may result in elevated quinidine plasma concentrations and associated ECG changes, including a prolonged QT interval. In addition, several cases of tinnitus and decreased hearing have been reported during concomitant therapy (Kaukonen *et al.*, 1997). The concurrent administration of itraconazole and quinidine is contraindicated (Prod Info Sporanox(R), 2000).

8.14 Vinca alkaloids

The concomitant administration of itraconazole and vincristine has been associated with an increased incidence of neurotoxicity in children and adults with acute lymphoblastic leukemia (ALL). Possible mechanisms for this interaction include the inhibition of cytochrome P450 enzymes by itraconazole that are involved in vinca alkaloid metabolism. Itraconazole may also inhibit the P-glycoprotein efflux pump, which is responsible for increasing the excretion of vinca alkaloids. Death has been reported in a patient receiving the combination vinorelbine and itraconazole (Bosque, 2001). Caution and close monitoring should be utilized in patients who receive itraconazole and vinca alkaloids concurrently (Sporanox(R), 2000; Bohme *et al.*, 1995; Murphy *et al.*, 1995).

8.15 Food

The absorption of oral itraconazole in capsule form is influenced by the presence of food. When taken on an empty stomach, the systemic bioavailability of itraconazole capsules is approximately 40%, whereas a bioavailability of 102% has been

reported when administered with meals, or shortly after (Van Peer *et al.*, 1989; Grant and Clissold, 1989). The better absorption with food appears related to greater solubility (Van Peer *et al.*, 1989). It is recommended that itraconazole capsules be taken with meals to assure optimal oral systemic availability (Van Peer *et al.*, 1989; Grant and Clissold, 1989; Prod Info Sporanox(R), 1999). Conversely, the oral solution form of itraconazole has increased bioavailability when administered in the fasting state, making this dosage form ideal for patients who have difficulty swallowing capsules or whose ability to absorb itraconazole may be altered (Barone *et al.*, 1998).

9 Factors Affecting the Pharmacokinetics of Itraconazole

9.1 Influence of Food Intake

Early studies in healthy volunteers showed that oral intake of itraconazole on an empty stomach results in lower systemic availability than if the drug is administered with food. Barone *et al.* (1998) found no difference in the rate of itraconazole absorption from capsules, but showed that a meal enhanced the amount of itraconazole absorbed by 40%. The extent of hydroxy-itraconazole formation was not affected by food. In randomised studies in patients given a single dose of itraconazole 100 mg (the capsule formulation), drug absorption was almost twice as high after a high-fat meal, than when the drug was given on an empty stomach. Gastric pH was significantly ower after the meal, than before it. Absorption of the lipophilic weak base itraconazole seemed to be promoted by low stomach pH and a high-fat meal. In a study on healthy volunteers it was observed that the AUC values for itraconazole increased by 80% when administered with acidic beverage(Coca-Cola, pH=2.5), versus water (Poirier and Cheymol, 1998).

9.2 Renal Insufficiency

The effect of renal impairment on itraconazole pharmacokinetics was assessed in a single 200mg dose study, on a small group of uraemic patients with various clinical conditions. The pharmacokinetics of the drug in undialysed and haemodialysed

patients were similar and parameters were in the same range as those determined in healthy individuals, but patient groups were small. (Poirier and Cheymol, 1998).

Dosage adjustments of itraconazole are not required in patients with renal impairment (Boelaert *et al.*, 1988).

9.3 Hepatic Insufficiency

Very little in known about the effects of hepatic dysfunction on the pharmacokinetic properties of itraconazole, In 12 patients with cirrhosis given a single dose of itraconazole 100 mg, the $t_{1/2}$ was 37 $\pm$18 hours (mean $\pm$ SD). This was slightly longer than that observed in the control group. (Poirier and Cheymol, 1998).

Peak plasma levels, areas under the concentration time-curve (AUC), and elimination half-lives of oral itraconazole in patients with cirrhosis were similar to those reported in healthy volunteers in 1 small study (Grant and Clissold, 1989), suggesting no need for dose adjustments. However, itraconazole is extensively metabolized in liver and further studies are needed to confirm these findings.

9.4 Immunocomprised Patients

Steady-state drug concentrations in immunocomprised patients are generally lower than those found in healthy volunteers. Smith *et al.* (1992) carried out a study in 8 patients with AIDS who were given itraconazole 200 mg/day as capsules each day after breakfast for 14 days. The steady-state plasma C_{max} on day 15 and AUC were about 50% lower than those reported in healthy individuals. A likely explanation is that absorption of itraconazole, which is dependent on gastric acidity, may be reduced as patients with AIDS experience gastric achlorhydria. There is no information available concerning albumin concentration and itraconazole protein binding in patients with AIDS, and the impact this may have on the pharmacokinetics of the drug.

The mean $\pm$SD apparent plasma clearance of itraconazole (CL/F) calculated from results obtained at steady state in patients with AIDS receiving 200 mg/day and patients with leukaemia treated with 100 mg/day was highly variable:45.6 $\pm$ 22.8 L/h and 78.78 $\pm$ 6.9 L/h, respectively.

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 drud-drug interaction occurs only when 2 or more drugs compete for the same enzyme and when the metabolic reaction catalysed 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

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

(v) cleavage of the O-O bond

(vi) substrate oxygenation

(vii) product release.

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 can not only bind to the prosthetic haem iron, but also to the lipophilic region of the protein. Inhibitors that simultaneously bind to both regions are inherently more potent inhibitors. The potency of an 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 promotor, 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, coadministration 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 *N*-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. In 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 is 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, the reduction in drug concentration can be circumvented by increasing the drug dosage. 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).

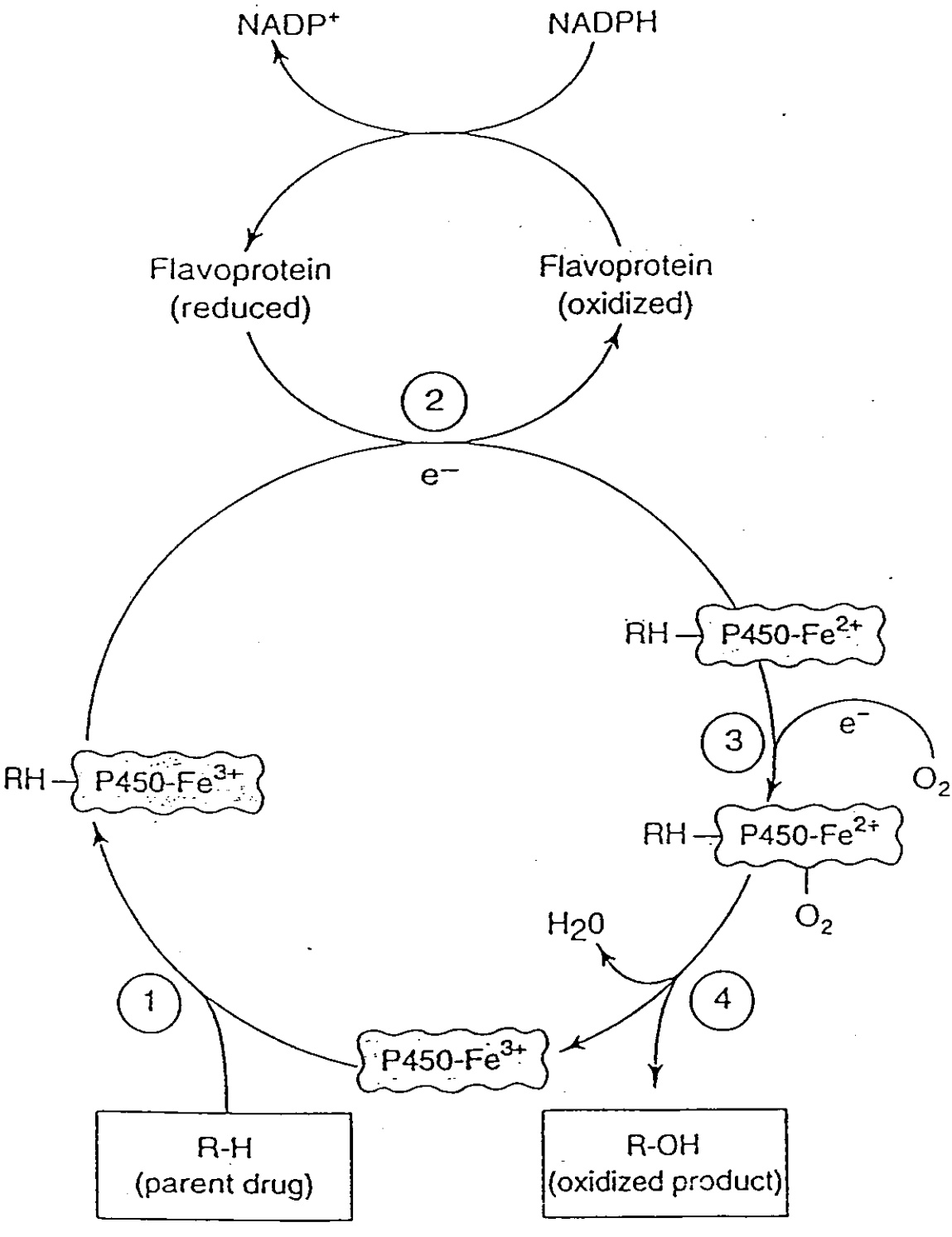

Figure 5 Cytochrome P-450 cycle in drug oxidations (Correia, 1998 :52)

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 *pfmdr*, 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-transmem-brane 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 cells 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 1a-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 *mdr1a*-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 an 11 fold increase in C_{max} for orally administered paclitaxel was

observed in the *mdr1a*-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. Coadministration 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 coadministered drug. This increase is greater than that observed in the knockout mice and is likely due to inhibition of CYP3A in the intestine and liver, suggesting a combined role of P-gp and CYP3A in limiting oral bioavailability of substrate drugs.

Kim and co-workers (1998) recently observed that P-gp also imits the oral bioavailability of the HIV protease Inhibitors indinavir, nelfinavir, and saquinavir, which suggests more effective treatment of this disease may be achieved by coadministration of a P-gp inhibitor with these agents. Administration of these protease inhibitors to *mdr1a*-deficient mice resulted in two- to five-fold higher plasma concentrations and a seven- to 36-fold increased brain accumulation of the drugs. These authors suggest that targeted inhibition of P-gp would result in higher protease inhibitor concentrations and more effective therapy.

The Caco-2 human intestinal cell line is a well-studied model for assessing drug absorption and investigation of mechanisms that affect oral bioavailability. Hunter and co-workers (1993) used immunofluorescence with the MRK16 antibody to demonstrate apical expression of P-gp in these cells. Using specialized dual-chamber tissue culture dishes, these authors observed transporter-mediated, directional, and saturable secretion of vinblastine from the basolateral toward the apical side of Caco-2 mono layers. This transport was inhibited by several P-gp modulators such as verapamil, MRK16, taxotere, 1,9-dideoxyforskolin, and nifedipine. Furthermore, this inhibition led to a dose-dependent increase in vinblastine absorptive flux. Similarly, P-gp mediated time and concentration dependent polarized efflux of CsA was observed in Caco-2 cells and was suggested to be a key physiologic determinant of CsA oral bioavailability. These

data provided early support for the hypothesis that P-gp, located at the tip of the intestinal villus, is a barrier for drug absorption.

Intestinal absorption of β-adrenoreceptor antagonists (β-blockers) is variable and has been shown to be somewhat dependent on lipophilicity. Absorption of one such β-blockers, celiprolol, increases at high doses and is nonlinear in humans. Studies with Caco-2 cells show that celiprolol is actively and saturably effluxed but passively and nonsaturably absorbed, suggesting the involvement of an active transport mechanism. Celiprolol basolateral to apical (secretory) transport was inhibited by the P-gp substrate vinblastine as well as the P-gp reversal agents verapamil and quinidine. These data suggest that this transporter is involved in celiprolol absorption. Similarly, basolateral to apical transport of acebutolol is two-fold greater than in the reverse direction. Intestinal absorption of acebutolol is increased 2.6-fold in the presence of CsA. Combined, these data as well as numerous additional investigations clearly demonstrate that P-gp is one factor important in determining drug absorption and elimination.

Recent studies have demonstrated that interaction between intestinal drug metabolism by cytochrome P450 3A and P-gp-mediated transport may contribute to the poor oral bioavailability and high interpatient and intrapatient variability in absorption of drugs. The liver has been classically viewed as the primary site of drug metabolism; recently, however, it has been recognized that a significant amount of drug metabolism occurs in the intestine and is mediated by CYP3A. Although the intestine does not quantitatively have as much CYP3A as the liver, the enzyme is located in the differentiated villus cells, which are the site of drug absorption. Greater than 50% of clinically important drugs are metabolized by CYP3A; thus, its location in the intestine suggests a critical role for it in oral drug bioavailability. Recently, a striking overlap between the substrates for P-gp and cytochrome P450 3A family members has been observed. Simultaneous expression of these proteins in 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 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 polarised 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_i 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 polarised 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 dificient 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 fo 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 cause by itraconazole coadministrtion, 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 have 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 [in preparation], can be increased by concomitant administration of ketoconazole, a potent CYP3A inhibitor (K_i approximately 1 μM) and an intermediate inhibitor of P-gp (K_i approximately 120 μM) (Benet *et al.*, 1999).

Conversely, concomitant administration of rifampin, a potent inducer 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 suggest 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-mediated

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 and associates 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 (Palkama *et al.*, 1999).

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 and associates (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.