

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) was jointly developed by Merck E and Bayer AG after it was identified in 1972 from a group of heterocyclic pyrazino-isoquinoline derivatives and found to have unusually broad anthelmintic activity (Pearson and Guerrant, 1983).

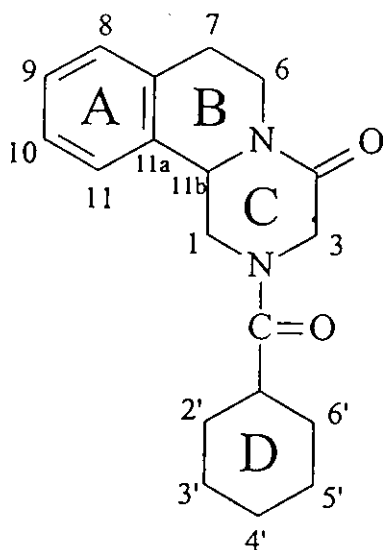


Figure 1 Structural Formula of Praziquantel

(Giorgi *et al.*, 2001)

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)

1. Pharmacodynamic Properties

1.1 Mechanism of Action

After rapid and reversible uptake, praziquantel has two major effects on 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 for example, in the rapid shift of adult *schistosomes* from the mesenteric veins to the liver or in the expulsion of intestinal cestodes into the environment. At slightly higher therapeutic concentration, praziquantel causes tegumental damage, which activates host defense mechanisms and results in destruction of the worms. Comparisons of stage-specific susceptibility of *S. mansoni* to praziquantel *in vitro* and *in vivo* indicate that the clinical efficacy of this drug correlated better with its tegumental action.

Membranes of affected helminths appear to be the primary target for praziquantel action, but the molecular mechanism is unknown. The compound causes increased membrane permeability to certain monovalent and divalent

cations, particularly Ca^{2+} . Both drug-induced muscular contraction and tegumental damage of *S. mansoni* require extracellular Ca^{2+} , but praziquantel acts differently than do K^+ , Ca^{2+} ionophores, that affect mammalian membranes. Praziquantel also produces a variety of biochemical changes, but most appear to be secondary to its primary tegumental action (Tracy and Webster, 2001).

1.2 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 $0.3 \mu\text{g/ml}$. 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 forms are killed; thus, praziquantel has a prophylactic effect (Goldsmith, 2001).

1.3 Resistance

As yet there is no confirmed report of anthelmintic drug resistance in a soil-transmitted nematode infection in humans. The situation with praziquantel

was believed to be good until 2 recent developments: first, of some concern are reports describing apparent low cure rates in infected patients in Senegal, although the data may also reflect the high rates of reinfection in this naive population. Second, laboratory evidence of diminished susceptibility to praziquantel in a schistosome isolated from the same area in Senegal is evidence of the existence of a resistance genotype, but not necessarily evidence for its selection.

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 *et al.*, 1997).

2. Pharmacokinetic Properties

2.1 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 $\mu\text{g/ml}$ are achieved in the systemic circulation 1 to 3 hours after administration of therapeutic doses (King and Mahmoud, 1989).

Although there is considerable variability 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 presence of food affects the pharmacokinetic properties 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.

2.2 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).

2.3 Elimination

In human, oral praziquantel undergoes extensive first pass hepatic biotransformation in to 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-hydroxypraziquantel 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).

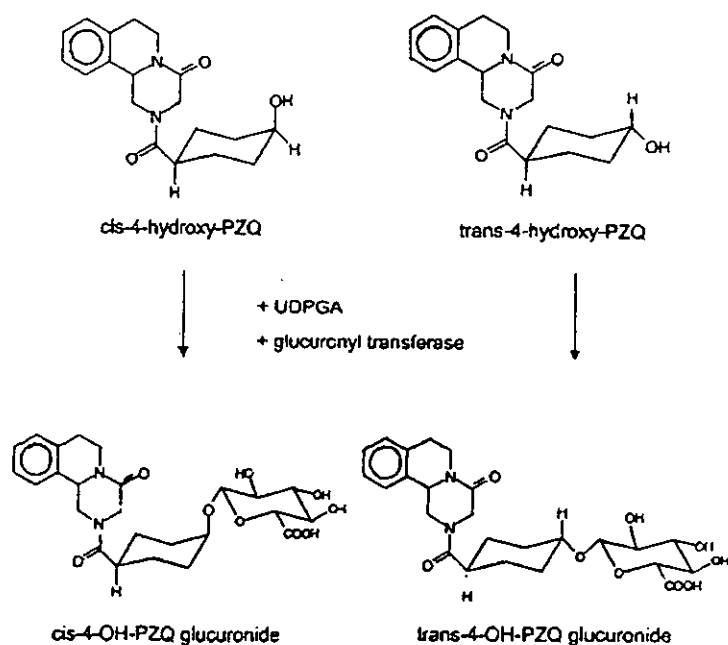


Figure 2. Conjugation of *trans*- and *cis*-4-hydroxypraziquantel with uridine-5'-diphosphoglucuronide acid (UDPGA) and microsomes containing glucuronyl transferase (Meier and Blaschke, 2000).

3. Pharmacokinetics in Various Pathophysiological States

3.1 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 significantly greater (Na-Bangchang *et al.*, 1993).

3.2 Schistosomiasis Patients

The pharmacokinetic of praziquantel were investigated in Sudanese patients with hepatosplenic schistosomiasis and in healthy volunteers. In patients, higher plasma levels of praziquantel were noted ($P < 0.05$ 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 al.*, 1990).

3.3 Neurocysticercosis Patients

Praziquantel was measured in plasma in 29 patients with neurocysticercosis. Mean level of praziquantel was 1.64 $\mu\text{g/ml}$ 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).

3.4 Schistosomiasis Patients with and without Liver Cell Failure

The pharmacokinetic and therapeutic efficacy of praziquantel were studied in 40 patients with *Schistosoma mansoni* and various degrees of hepatic dysfunction. Every patient was treated with 40 mg/kg of praziquantel as a single oral dose. The pharmacokinetic 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).

3.5 *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 *Schistosoma japonicum* 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).

3.6 *Schistosoma haematobium*-Infected Subjects

The kinetics of praziquantel was studied in normal and *Schistosoma haematobium*-infected Ghanaian subjects. There was a wide interindividual variation in $t_{1/2}$, C_{max} , T_{max} , $AUC_{(0-8\text{ h})}$ and urinary recovery. No difference were noted between the two groups with regard to T_{max} , $AUC_{(0-8\text{ h})}$ and $t_{1/2}$. Mean C_{max} was higher in the patients than in the control subjects. The 8-hr urinary recovery of praziquantel was higher in the subjects with urinary schistosomiasis. The amount of praziquantel excreted unchanged in urine was

0.0052 ± 0.0027 % of the dose for the control subjects and 0.0054 ± 0.0027 % for the patients (Ofori-Adjei *et al.*, 1988).

3.7 Schistosomiasis Patients with Chronic Renal Failure

The pharmacokinetic of praziquantel in a uraemic patient, infected with *Schistosoma haematobium*, undergoing haemodialysis, was studied by repeated analyses of serum, urine and dialysis fluid. The data do not suggest that the kinetics of praziquantel in blood were changed in any way by the underlying kidney disease. Renal elimination of unmetabolized praziquantel was changed only slightly. The results indicate the possibility of treating advanced with *S. haematobium* with the normally recommended doses (Pehrson *et al.*, 1983).

4. Clinical Uses

4.1 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).

4.2 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 daily for 1 day for opisthorchiasis and 2 days for clonorchiasis infections results in nearly 100% cure rates. (Goldsmith, 2001).

4.3 Paragonimiasis

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

4.4 Taeniasis and Diphylobothriasis

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 single 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 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).

4.5 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).

4.6 *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).

4.7 Other Parasites

Limited trials at a dosage of 25 mg/kg three 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).

5. Adverse Reactions

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 corticosteroids 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 hypertension 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).

6. Toxicity

In humans, no major alterations in biochemical or hematological tests have been described. Transient elevations in liver function tests and, rarely, minor electrocardiographic changes may occur, but no significant damage to vital organs have been reported. In severe liver dysfunction, plasma levels rise.

In experimental animals, no effects are seen until doses approximately 100 times the therapeutic range are reached; signs of central nervous system toxicity are then noted.

Although a wide variety of mutagenicity, carcinogenicity, embryotoxicity, and teratogenicity studies have indicated that praziquantel dose not present these risks, new findings suggest a potential for genotoxicity and carcinogenicity; the subject needs further assessment (Goldsmith, 2001).

7. Contraindications and Cautions

The only specific contraindication is ocular cysticercosis; parasite destruction in the eye may cause irreparable damage. Some workers also caution against use of the drug in spinal neurocysticercosis. In cysticercosis-endemic areas, patients treated with praziquantel for conditions other than cysticercosis should be observed carefully in hospital for about 48 hours after completion of treatment. The drug can be used in the presence of liver impairment, but a reduction in dose may be necessary. Although safety of the drug in children under age 4 years is not established, no pediatric-specific problems have been documented.

Because the drug induces dizziness and drowsiness, patients should not drive and should be warned if their work requires physical coordination or alertness.

The drug should preferably not be taken during pregnancy; an increase in abortion rate was found in rats treated with three times the human dose. In lactating woman, although praziquantel appears in milk at about one-fourth of the plasma levels, the drug may be given to the mother provided the infants is not nursed on the day of treatment and for 3 subsequent days.

Patients should be cautioned against chewing the bitter-tasting drug; the retching that results can become a special problem in treatment of *T. solium* infection because regurgitation of segments might results, which could be hazardous (Goldsmith, 2001).

8. Drug Interaction

8.1 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).

8.2 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).

8.3 Cimetidine

A patient had neurocysticercosis complicated by a seizure 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/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 half-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 at all time intervals. The difference was statistically significant at 3, 4, 6 and 8 hours following treatment. On the other hand, coadministration of cimetidine with brand 2 (Distocide) 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 K_e 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.

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).

8.4 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 reactions (Vazquez *et al.*, 1987).

8.5 Antiepileptics Drugs

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 pharmacokinetic drug interactions of the concomitant therapy with antiepileptic drugs (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).

Bittencourt *et al.* (1992) showed that carbamazepine and phenytoin significantly decreased concentrations of praziquantel, due to increased clearance secondary to induction of first pass-liver metabolism. The magnitude of the decrease is surprisingly high and may be responsible for failures of treatment.

8.6 Chloroquine

Masimirembwa *et al.* (1994) observed that chloroquine decreased the bioavailability of praziquantel and reduced its maximum serum concentrations to a significant extent in rats and in human. The clearance was increased to a statistically significant extent in rats but not in human because of the wide interindividual variation. The effect of chloroquine on praziquantel pharmacokinetics was unexpected since drug that inhibit hepatic drug metabolism usually increase the bioavailability of praziquantel. They found that chloroquine inhibits non-competitively the metabolism of praziquantel to its major metabolite, 4-hydroxy- praziquantel, with a K_i of 1.65 mM in rat hepatic microsomes. Maximum concentrations attained by chloroquine in serum, however, are low compared to the K_i value and significant inhibition is therefore unlikely *in vivo*. The explanation for chloroquine' effect on the pharmacokinetics of praziquantel may be due to other effect of chloroquine

rather than to a direct effect on drug-metabolizing enzymes.

8.7 Albendazole

Homeida *et al.* (1994) demonstrated that praziquantel pharmacokinetics were not affected by coadministration of albendazole, but the AUC of albendazole sulphoxide (the active metabolite of albendazole) increased 4.5 folds when administered with praziquantel. The reasons for the increases in the AUC of albendazole sulphoxide, are even more difficult to explain. In such an acute study, it is unlikely that the praziquantel could have induced P450 activity and thereby increased the rate of biotransformation.

Rifampicin

Rifampicin (Figure 3) was first introduced in 1963 (Mcnicol, *et al.*, 1995). The rifamycins are a group of structurally similar, complex macrocyclic antibiotics produced by *Streptomyces mediterranei*; rifampicin is a semisynthetic derivative of this rifamycin B (Mandell and Sandle, 1991).

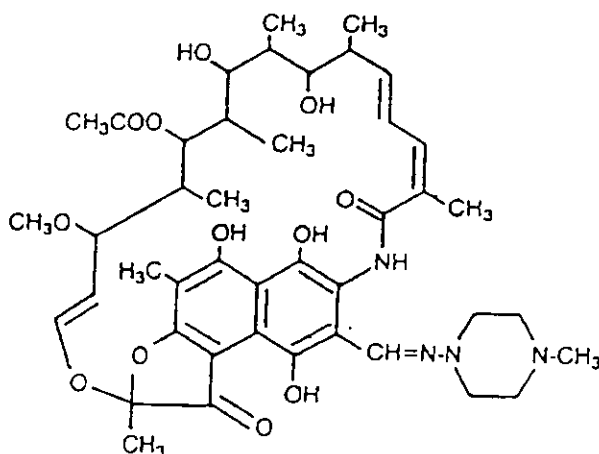


Figure 3 Structural formula of rifampicin

Chemical and Physical Properties

Chemical structure : $C_{43}H_{58}N_4O_{12}$

Molecular weight : 822.96

pKa :

4-hydroxy group : 1.7

3-piperazine nitrogen group : 7.9

(Windholz *et al.*, 1976)

Solubility :

in water, acetone, alcohol and ether : slightly

in chloroform : freely

(Reynolds, 1993)

1. Pharmacodynamic Properties

1.1 Mechanism of Action

Rifampicin inhibits DNA-dependent RNA polymerase of mycobacteria and other microorganisms by forming a stable drug-enzyme complex, leading to suppression of initiation of chain formation (but not chain elongation) in RNA synthesis. More specifically, the β subunit of this complex enzyme is the site of action of the drug, although rifampicin binds only to the holoenzyme. Nuclear RNA polymerase from a variety of eukaryotic cells does not bind rifampicin, and RNA synthesis is correspondingly unaffected. While rifampicin can inhibit RNA synthesis in mammalian mitochondria, considerably higher concentrations of the drug are required than for the inhibition of the bacterial enzyme. Rifampicin is bactericidal for both intracellular and extracellular microorganisms (William and Petri, 2001).

1.2 Antibacterial Activity

Rifampicin inhibits the growth of most gram-positive bacteria as well as many gram-negative microorganisms such as *Escherichia coli*, *Pseudomonas*, *indole-positive* and *indole-negative Proteus*, and *Klebsiella*. Rifampicin is very active against *Staphylococcus aureus* and *coagulase-negative staphylococci*; bactericidal concentrations range from 3 to 12 ng/ml. The drug also is highly active against *Neisseria meningitidis* and *Haemophilus influenzae*; minimal inhibitory concentrations range from 0.1 to 0.8 $\mu\text{g/ml}$. Rifampicin is very inhibitory to *Legionella* species in cell culture and in animal models (William and Petri, 2001).

Rifampicin in concentrations of 0.005 to 0.2 $\mu\text{g/ml}$ inhibits the growth of *M. tuberculosis in vitro*. Among nontuberculous mycobacteria,

M. kansasii is inhibited by 0.25 to 1 µg/ml. The majority of stains of *M. scrofulaceum*, *M. intracellulare*, and *M. avium* are suppressed by concentrations of 4 µg/ml, but certain strains may be resistant to 16 µg/ml. *M. fortuitum* is highly resistant to the drug. Rifampicin increases the *in vitro* activity of streptomycin and isoniazid, but not that of ethambutol, against *M. tuberculosis* (William and Petri, 2001).

1.3 Resistance

Microorganisms, including mycobacteria, may develop resistance to rifampicin rapidly *in vitro* as a one-step process, and one of every 10^7 to 10^8 tubercle bacilli is resistant to the drug. Resistance in most cases is due to mutations between codons 507 and 553 of the polymerase *rpoB* gene. This also appears to be the case *in vivo*, and therefore the antibiotic must not be used alone in the chemotherapy of tuberculosis. When rifampicin was used for eradication of the meningococcal carrier state, failures was due to the appearance of drug-resistant bacteria after treatment for as little as 2 days. Microbial resistance to rifampicin is due to an alteration of the target of this drug, DNA-dependent RNA polymerase. Certain rifampicin-resistance bacterial mutants have decreased virulence. Tuberculosis caused by rifampicin-resistant mycobacteria has been described in patients who had not received prior chemotherapy, but this is very rare (usually less than 1%) (William and Petri, 2001).

2. Pharmacokinetic Properties

2.1 Absorption

The oral administration of rifampicin produces peak concentrations in

plasma in 2 to 4 hours ; after ingestion of 600 mg, this value is about 7 $\mu\text{g/ml}$, but there is considerable variability. Aminosalicyclic acid may delay the absorption of rifampicin, and adequate plasma concentrations may not be reached. If these agents are used concurrently, they should be given separately at an interval of 8 to 12 hours (William and Petri, 2001).

Food has been demonstrated to cause a 1 to 2 hours delay in the T_{max} and a reduction in the C_{max} and AUC of rifampicin. In studies in which rifampicin was given in combination with other antituberculosis drugs, some notable effects upon absorption and bioavailability have been reported. Rifampicin dose not influence the serum concentrations of isoniazid but conflicting reports have been published on the effect of isoniazid on the former. On the other hand, para-aminosalicylic acid was found to delay the T_{max} of rifampicin from 2 to 4 hours, reduce its C_{max} from 8.0 to 3.8 $\mu\text{g/ml}$, and decrease the AUC by approximately 50% (Boman *et al.*, 1974). This effect was thought to be due to impairment of gastrointestinal absorption of rifampicin by either alteration of the physicochemical properties of the mucosa by para-aminosalicylic acid, or a decrease in gastric emptying rate with an increased intestinal transit time. In other studies, simultaneous administration of para-aminosalicylic acid and rifampicin has also been shown to decrease the C_{max} of rifampicin due to the absorption of the latter onto the bentonite content of the para-aminosalicylic acid granules, thereby leading to decreased intestinal absorption of rifampicin (Acocella and Conti 1980 ; Boman *et al.*, 1971). Excipients such as talcum and kaolin have also been suspected to reduce gastrointestinal absorption of rifampicin. In addition, food may decrease its absorption (Sieglar *et al.*, 1974), and decreased serum

concentrations of rifampicin have been observed after prolonged administration (Holdiness, 1984).

2.2 Distribution

Rifampicin is widely distributed with good penetration of all normal tissues including bone and serous fluid. It does not penetrate the normal blood brain barrier, but penetrates well when there is inflammation. The tissue levels attained are about 100 times higher than the MIC for sensitive strains (Mcnicol *et al.*, 1995). At physiological pH, only 25% of the compound of rifampicin are ionized, but the molecule, as a whole is lipid soluble. The Vd (55.5 L) has been demonstrated to be independent of infusion rates in 12 tuberculosis patients. (Holdiness, 1984). Rifampicin is relatively highly protein-bound. In normal subjects protein binding is approximately 80% suggested that the γ -globulin may be the main serum binding proteins (Holdiness, 1984 ; Chambers and Jawetz, 1998).

Rifampicin is distributed throughout the body and is present in effective concentrations in many organs and body fluids, including the CSF. This is perhaps best exemplified by the fact that the drug may impart an orange-red color to the urine, feces, saliva, sputum, tears and sweat; patients should be so warned. (William and Petri, 2001).

2.3 Metabolism

Rifampicin is slowly acetylated in the liver and the unchanged drug together with acetylrifampicin is excreted in the bile. There is a significant enterohepatic circulation of rifampicin and very high concentrations are achieved in bile. Desacetylrifampicin is not reabsorbed and most of the drug is excreted in the feces in this form. It is also excreted in the urine where very

high concentrations are attained. The pathway of rifampicin metabolism, and metabolic derivatives are illustrated in Figure 4.

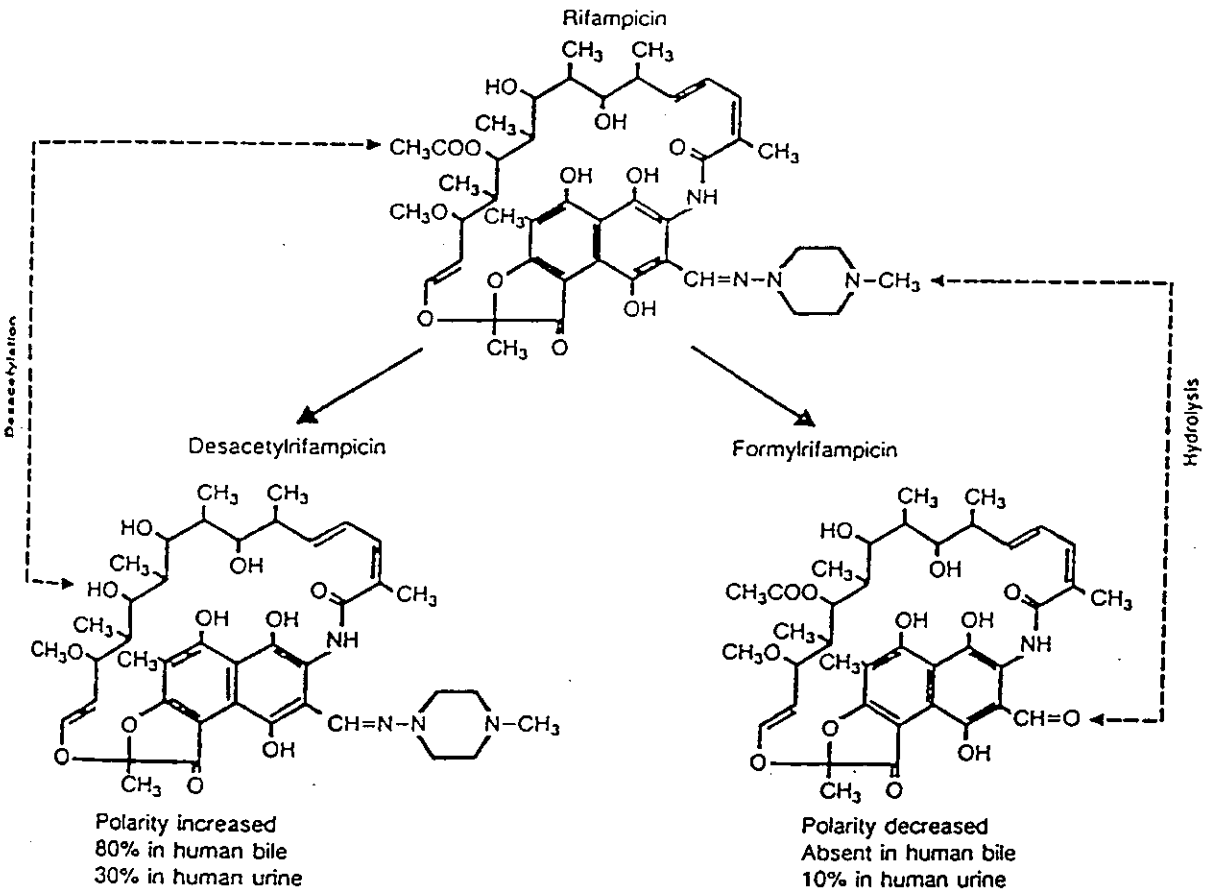


Figure 4 Principal metabolic derivatives of rifampicin in man, polarity and percentage recovery in bile and urine (Holdiness, 1984).

In figure 4, it presents the metabolic routes of rifampicin in man, the major pathway bring deacetylation at the C-25 position, most probably in the liver. This results in a more water-soluble compound with an increased capacity for biliary excretion. In a separate metabolism pathway, hydrolysis yields a 3-formyl-rifampicin derivative. The deacetylated from accounts for approximately 80% of the microbiological activity in human bile and its rate of transfer into bile is 10 to 20 times greater than that of the parent compound. The deacetylating enzymes are thought to be located in the smooth

endoplasmic reticulum of the hepatocytes as evidenced by the proliferation of smooth endoplasmic reticulum with continued administration of rifampicin (demonstrated by electron microscopic examination). Along with these ultrastructure changes are concomitant increases in hepatic cytochrome P450, β -glucuronidase, paranitrophenolglucuronyltransferase, β -N-acetylglucuronidase, β -N-acetylglucosaminidase, and corticosteroid hydroxylase.

It has also been demonstrated recently that 15 to 20% of deacetylrifampicin is converted to a glucuronide, this process being commonly catalyzed by smooth endoplasmic reticulum enzymes (Holdiness, 1984).

2.4 Excretion

The half-life of rifampicin varies from 1.5 to 5 hours and is increased in the presence of hepatic dysfunction ; it may be decreased in patients receiving isoniazid concurrently who are slow inactivators of this drug. The half-life of rifampicin is progressively shortened by about 40% during the first 14 days of treatment, owing to induction of hepatic microsomal enzymes with acceleration of deacetylation of the drug. Up to 30% of the dose of the drug is excreted in the urine and 60% to 65% in the feces; less than half of this may be unaltered antibiotic. Adjustment of dosage is not necessary in patients with impaired renal function (William and Petri, 2001).

3. Pharmacokinetic in Various Pathophysiological States

3.1 Renal Disease

Rifampicin can also be administered in full therapeutic doses to patients with severely impaired renal function without toxic effects. Studies in

the early 1970s with 300 mg rifampicin found little difference between normal patients and those with severe renal insufficiency. However, when the dose was increased to 600 mg 34 to 40% increases in AUC and $t_{1/2}$ were noted probably due to oversaturation of the hepatic metabolizing capacity as well as decreased renal clearance.

Reduced renal clearance of rifampicin seems to be compensated by enhanced biliary elimination, and a dose of 600 mg/day does not appear to need reduction in patients with limited renal function. Studies of peritoneal and haemodialysis patients receiving rifampicin also indicate that the antibiotic is dialysable without difficulty (Holdiness, 1984).

3.2 Liver Disease

Numerous studies have demonstrated increased elimination half-life in patients with liver diseases given oral doses of 300 to 900 mg rifampicin. Following a lower dose of 450 mg, serum half-life of 2.5, 6.0 and 6.5 hours have been observed in normal, cirrhotic and hepatitis subjects, respectively. In one study slight increases in the AUC of rifampicin were observed in patients with chronic liver disease compared with normal subjects, the AUC_{0-12} values in the 2 groups after 7 days of treatment with 600 mg rifampicin daily being 55.1 $\mu\text{g/ml.h}$ (normals) and 105.2 $\mu\text{g/ml.h}$ (liver disease). Associated with this was a small increase in serum $t_{1/2}$ in the chronic liver disease patients (3.2 h vs. 1.8 h in the normal subjects on the seventh day of treatment). Despite the longer serum $t_{1/2}$ values, no marked differences in total urinary excretion were noted in the above studies, suggesting that patients with various liver diseases have the same ability to metabolize rifampicin as normal individuals. However, the increased serum concentrations of rifampicin could give rise to

increased bilirubin levels as a result of competition for common biliary excretion mechanisms. Thus in the presence of severe hepatic disease, there may be a need to reduce the dosage of rifampicin and monitor serum concentrations in order to avoid the occurrence of hyperbilirubinaemia (Holdiness, 1984).

3.3 Infants and Children

Reduced serum concentrations of rifampicin in comparison with adults have been noted in neonates and children up to 18 months of age given equivalent doses in terms of mg/kg bodyweight. Mean peak serum concentrations of 3.5 to 4.2, 7.1 and 9.6 to 12.0 $\mu\text{g/ml}$ after preprandial administration of single rifampicin doses of 10, 15 and 20 mg/kg bodyweight, respectively, have been recorded in children. Peak serum concentrations of rifampicin in children were found to be approximately one-third to one-tenth those of adults given a similar dose based on bodyweight; this difference is possibly due to the large total body compartments in infants (Holdiness, 1984).

In new born less than 3 days of age, peak serum concentrations after a 10 mg/kg dose were reached up to 8 hours after dosing and were followed by comparatively slow elimination. This is possibly due to the undeveloped hepatic capacity for the drug, and a high gastric pH. Following repeated administration of dose of 10 mg/kg in newborns, accumulation of the drug has been noted. A study of the urinary excretion of rifampicin in newborns (aged less than 3 days) and young children (age 4 to 18 months) given doses of 10 mg/kg showed that 37% of the dose was recovered in urine in the first 12 hours in the newborns compared with 2.5% of the dose in the older children (Holdiness, 1984).

3.4 Elderly Patients

Recently, the pharmacokinetic of rifampicin have been studied in 6 elderly individuals (age 78 to 95 years) after single oral doses of 10 mg/kg. The C_{\max} and $t_{1/2}$ of rifampicin were $8.83 \pm 1.72 \mu\text{g/ml}$ and 4.09 ± 2.59 hours, respectively, which are comparable to those reported in younger adults. The same also applies to the C_{\max} ($1.93 \pm 0.53 \mu\text{g/ml}$) and the $t_{1/2}$ (4.65 ± 2.61 h) values of deacetylrifampicin. However, the renal clearance of rifampicin (7.5 ± 3.6 ml/min) and deacetylrifampicin (14.6 ± 2.7 ml/min) during a 24-hour period were lower than those observed in younger individuals (rifampicin, 30 ± 7.6 ml/min ; deacetylrifampicin, 22.5 ± 10 ml/min). The authors suggested that since the drug is eliminated via the liver to such an extent that serum concentrations are the same as in younger adults, for therapeutic purposes the metabolism of rifampicin may be globally considered as unaltered in elderly patients (Holdiness, 1984).

3.5 Pregnancy

There is no convincing evidence of teratogenicity in the human but high doses are teratogenic in rats and mice. It was formerly recommended that rifampicin should be avoided during the first 3 months of pregnancy, but in the absence of firm evidence of teratogenicity in humans this recommendation has been abandoned. Particularly when disease is extensive or the patient is ill, it is generally agreed that active tuberculosis is a much more serious threat to the mother and fetus than the use of rifampicin, which is an important component of the most effective antituberculosis regimens. Rifampicin is excreted in breast milk. This dose not appears to be associated with clinically important problems and is not a contraindication to breast feeding (Mcnicol *et al.*, 1995).

4. Adverse Reactions

Rifampicin generally is well tolerated. When given in usual doses, fewer than 4% of patients with tuberculosis have significant adverse reaction ; the most common are rash (0.8%), fever (0.5%) and nausea and vomiting (1.5%). Rarely, hepatitis and deaths due to liver failure have been observed in patients who received other hepatotoxic agents in addition to rifampicin or who had preexisting liver disease. Hepatitis from rifampicin rarely occurs in patients with normal hepatic function; likewise, the combination of isoniazid and rifampicin appears generally safe in such patients. However, chronic liver disease, alcoholism and old age appear to increase the incidence of severe hepatic problems when rifampicin is given alone or concurrently with isoniazid (William and Petri, 2001).

Administration of rifampicin on an intermittent schedule (less than twice weekly) and/or daily doses of 1,200 mg or greater is associated with frequent side effects, and the drug should not be used in this manner. A flu-like syndrome with fever, chill and myalgias develops in 20% of patients so treated. The syndrome also may include eosinophilia, interstitial nephritis, acute tubular necrosis, thrombocytopenia, hemolytic anemia and shock (William and Petri, 2001).

Because rifampicin is a potent inducer of hepatic microsomal enzymes, its administration results in a decreased half-life for a number of compounds, including HIV protease and nonnucleoside reverse transcriptase inhibitors, prednisone, digitoxin, digoxin, quinidine, disopyramide, mexiletine, tocainide, ketoconazole, propranolol, metoprolol, clofibrate, verapamil, methadone, cyclosporine, corticosteroids, oral anticoagulants, theophylline, barbiturates,

oral contraceptives, halothane, fluconazole and the sulfonylureas. The significant interaction between rifampicin and oral anticoagulants of the coumarin type leads to a decrease in efficacy of these agents. This effect appears about 5 to 8 days after rifampicin administration is started and persists for 5 to 7 days after it is stopped. The ability of rifampicin to enhance the catabolism of a variety of steroids leads to the decreased the effectiveness of oral contraceptives. The increased metabolism of Methadone has led to reports of precipitation of withdrawal syndromes. Rifampicin may reduce biliary excretion of contrast media used for visualization of the gallbladder (William and Petri, 2001).

5. Clinical Uses

5.1 Mycobacterial Infections

Rifampicin, usually 600 mg/day (10 mg/kg/day) orally, is administered together with INH, ethambutal, or another antituberculous drug in order to prevent emergence of drug-resistant mycobacteria. In some short-course therapies, 600 mg of rifampicin is given twice weekly. Rifampicin also is effective in some atypical mycobacterial infections and in leprosy when used together with a sulfone. Rifampicin is an alternative to INH prophylaxis for patients who are unable to take INH or who have had close contact with a case of active tuberculosis caused by an INH-resistant, rifampicin-susceptible strain (Chambers and Jawetz, 1998).

5.2 Other Indications

Rifampicin is used in a variety of other clinical situations. An oral dosage of 600 mg twice daily for 2 days can eliminate meningococcal

carriage. Rifampicin, 20 mg/kg/day for 4 days, is used as prophylaxis in contacts of children with *Haemophilus influenzae* type b disease. Rifampicin combined with a second agent is used to eradicate staphylococcal carriage. Rifampicin combination therapy is also indicated for treatment of serious staphylococcal infections such as osteomyelitis and prosthetic valve endocarditis. Rifampicin has been recommended also for use in combination with ceftriaxone or vancomycin in treatment of meningitis caused by highly penicillin-resistant strains of pneumococci (Chambers and Jawetz, 1998).

6. Drug Interactions

Rifampicin is used clinically in the treatment of tuberculosis, usually being administered for 4 to 12 months together with other antituberculosis agents or additional medications for an accompanying disease. It is a potent inducer of drug metabolism in humans and has been shown to produce a proliferation of smooth endoplasmic reticulum and to increase the cytochrome P450 content of human liver. There is a remarkable selectivity in the enzyme induction by rifampicin and not every drug metabolized by oxidation will be affected (Venkatesan, 1992).

6.1 Oral Anticoagulants

One of the first reported rifampicin interaction was with oral anticoagulants. Several groups of workers noticed that patients on long-term anticoagulants require an increase in the dose when rifampicin is coadministered.

O' Reilly (1974), who measured prothrombin time and plasma warfarin concentrations in 10 male volunteers after single oral and intravenous

dose of warfarin 1.5 mg/kg both before and during rifampicin treatment, observed a highly significant decrease in the mean areas under the prothrombin time-time curve and a corresponding decrease in plasma warfarin concentrations. That there was no significant alteration in the absorption of the anticoagulant by rifampicin suggests an induction of warfarin metabolizing enzymes.

In a subsequent study (1975), the same author administered warfarin 7.5 to 10 mg together with rifampicin 600 mg daily to 8 volunteers for 21 days, and noted a highly significant decrease of hypoprothrombinaemic effect and plasma warfarin concentrations associated with increased excretion of warfarin metabolites in urine and stool for the last 10 days of the study. Rifampicin withdrawal decreased the warfarin requirements by 50 to 60% (Venkatesan, 1992).

Heimark *et al.* (1987) have shown that the reduction in hypoprothrombinaemic response of warfarin by rifampicin is due to increased clearance of both warfarin enantiomers, thereby ruling out any regioselectivity or stereoselectivity of warfarin hydroxylating microsomal enzymes induced by rifampicin. A need for increased doses of acenocoumarol and phenprocoumon during concomitant administration of rifampicin has also been reported.

In cases of simultaneous rifampicin and anticoagulant therapy, the dosage of the latter should be adjusted on the basis of prothrombin time, which must be monitored especially when oral anticoagulant therapy is either initiated or terminated. In any case, the treatment of patients with anticoagulants is a highly individualized matter (Venkatesan, 1992).

6.2 Cardioactive Agents

Digoxin

Gault *et al.* (1984) described 2 dialysis-dependent patients who required substantial increases (34 to 100%) in digoxin doses to maintain therapeutic digoxin concentrations after the commencement of rifampicin 300 to 600 mg/day. When rifampicin therapy was stopped, therapeutic concentrations of digoxin were obtained with about 50% of the dose required previously.

The serum digoxin concentration of a patient with atrial fibrillation decreased from 2.9 to 1.7 $\mu\text{g/l}$ 4 days after initiation of rifampicin 600 mg/day. Further reductions occurred despite increasing the digoxin dose, while stopping rifampicin therapy (and reducing of the digoxin dose) resulted in serum digoxin concentrations of 1.6 and 2.6 $\mu\text{g/l}$ at 8 and 15 days after rifampicin therapy (Bussey *et al.*, 1984).

Greiner *et al.* (1999) suggested that the AUC of oral digoxin was significantly lower during rifampicin treatment. Renal clearance and half-life of digoxin were not altered by rifampicin. Rifampicin treatment increased intestinal P-glycoprotein content 3.5 ± 2.1 fold, which correlated with the AUC after oral digoxin. P-glycoprotein is a determinant of the disposition of digoxin. Concomitant administration of rifampicin reduced digoxin plasma concentrations substantially after oral administration.

Quinidine

A patient who responded well to quinidine 200 mg orally for syncope and palpitations associated with ventricular dysarrhythmia had a relapse within 1 week of initiation of therapy with rifampicin 600 mg/day concurrently with

ethambutal for coexistent tuberculosis. Despite increased quinidine dosage the peak plasma quinidine concentration decreased from 5 to 1 mg/L. the patient had therapeutic quinidine concentrations 1 week after rifampicin was replaced with isoniazid and the quinidine dose was increased from 1,200 to 1,600 mg/day. The AUC for quinidine was reduced by 4 to 6 folds, while its $t_{1/2}$ was enhanced 3 to 6 folds with concurrent oral administration of rifampicin to 8 volunteers receiving quinidine sulphate 6 mg/kg either intravenously or orally (Venkatesan, 1992).

Verapamil

A rifampicin-verapamil interaction has been reported in a 67-year-old patient with pulmonary tuberculosis treated with rifampicin and supraventricular arrhythmia uncontrolled with verapamil. A 4-fold increase in serum verapamil concentration after rifampicin was stopped, appeared to control the supraventricular arrhythmia (Barbarash, 1985).

Fromm *et al.* (1996) observed that rifampicin increased the systemic clearance of the active S-verapamil 1.3-fold. In contrast, rifampicin increased the apparent oral clearance of S-verapamil 32-fold and decreased its bioavailability 25-fold. Rifampicin altered the pharmacokinetics and the pharmacological effects of verapamil to a much greater extent after oral administration compared with intravenous administration.

Propafenone

Dilger *et al.* (2000) suggested that coadministration of rifampicin did not significantly alter the pharmacokinetic parameters of propafenone, *N*-desalkylpropafenone, or propafenone glucuronide after intravenous administration; only the AUC and A_e of 5-hydroxypropafenone gave a

difference. The effect of induction on pharmacokinetics of oral propafenone and its metabolites was significant. Bioavailability of propafenone decreased 87% in extensive metabolizers during induction, and correspondingly maximum QRS prolongation decreased by two thirds. Clearance to *N*-desalkylpropafenone, its conjugates, and propafenone glucuronide were enhanced significantly by rifampicin, indicating substantial enzyme induction. The cumulative urinary excretion of propafenone and its metabolites decreased during induction with rifampicin. It is interesting that pretreatment with rifampicin changed stereoselective phase 2 metabolism by increasing *R/S*-propafenone glucuronide concentration ratios both after intravenous and after oral propafenone. Rifampicin induced both phase 1 metabolism (*N*-desalkylation) and phase 2 metabolism (glucuronidation) of oral propafenone, resulting in a clinically relevant pharmacokinetic and pharmacodynamic drug interaction in the elderly.

6.3 Oral Contraceptives

Barditch-Crovo *et al.* (1999) showed that, the 14 days administration of 600 mg rifampicin daily (the recommended adult dose) produced a significant reduction in ethinyl estradiol trough concentrations, AUC, C_{\max} , and plasma $t_{1/2}$ and a significant increase in Cl/f . Rifampicin significantly reduced norethindrone trough levels, AUC, C_{\max} , and plasma $t_{1/2}$ and a significant increase in Cl/f .

A molecular basis has been reported for the rifampicin-oral contraceptive steroid interactions. Guengerich (1988) and Combalbert *et al.* (1989) showed that rifampicin induces a human liver cytochrome P450 which is a product of the P450 3A gene subfamily. The isozyme (P450 Nf, P450

3A3) is one of the major forms involved in the 2-hydroxylation of ethinylestradiol.

It is imperative to counsel woman who will receive oral contraceptives and rifampicin concurrently. As rifampicin clearly affects both estrogen and progesterone components of combined contraceptive steroids, no oral preparation is free from interaction. Since there are wide interindividual variations in response to rifampicin and it is usually given for a relatively short time, oral contraceptive steroids should not be given to woman taking rifampicin and alternative measures should be tried (Venkatesan, 1992).

6.4 Glucocorticoids

Prolonged administration of rifampicin increases the metabolism of many steroids, including cortisol and prednisolone. An increase in cortisone acetate replacement therapy from 50 to 100 mg/day was required when rifampicin 450 to 600 mg/day was given simultaneously to patients with Addison's disease. Patients with tuberculosis pericarditis, nephrotic syndrome or renal allografts also require increased glucocorticoid doses during concurrent administration of rifampicin (Venkatesan, 1992).

Lee *et al.* (1993) have studied the pharmacokinetic of prednisolone caused by co-administration or discontinuation of rifampicin in groups of 3 patients over a 1 month period of rifampicin co-treatment or after its withdrawal, revealed significant changes in the AUC, the total clearance, the non-renal clearance and the half-life. The changes in the pharmacokinetic parameters reached a 1.5 to 2 fold plateau after 2 weeks and the half-maximal effect was attained within 5 days. Neither the Vd nor the protein binding of prednisolone was significantly altered.

6.5 Hypoglycaemics

Tolbutamide

Syvalathi *et al.* (1974) observed a decrease in serum tolbutamide concentrations in tubercular patients who were receiving oral tolbutamide in conjunction with rifampicin. In a subsequent study by the same group (Syvalahti *et al.*, 1975), 9 patients with tuberculosis showed altered pharmacokinetics of the antidiabetic agent in response to intravenous dose of tolbutamide 1 g 4 weeks after starting therapy with rifampicin 450 to 600 mg/day. The $t_{1/2}$ of tolbutamide declined by 43 and 41% at 180 and 360 min, respectively, after concomitant rifampicin therapy.

Chlorpropamide

Diabetic control is poorer in patients on chlorpropamide during rifampicin coadministration. A 62-year-old patient with diabetes required an increase in his daily dosage from chlorpropamide 250 to 400 mg when rifampicin 600 mg/day was introduced as part of a tuberculosis regimen (Self and Morris, 1980).

Repaglinide

Rifampicin decreased the total AUC of repaglinide by 57% and C_{\max} of repaglinide by 41%. The elimination $t_{1/2}$ of repaglinide was shortened from 1.5 to 1.1 hours. The blood glucose decremental $AUC_{(0-3)}$ was reduced from 0.94 to -0.23 mmol/L.h, and the maximum decrease in blood glucose concentration from 1.6 to 1.0 mmol/L by rifampicin. Rifampicin considerably decreases the plasma concentrations of repaglinide and also reduces its effects. This interaction is probably caused by induction of the CYP3A4-mediated metabolism of repaglinide. (Niemi *et al.*, 2000).

Glyburide and Glipizide

Rifampicin decreased the AUC of glyburide by 39% and the C_{\max} by 22%. The elimination $t_{1/2}$ of glyburide was shortened from 2.0 to 1.7 hours by rifampicin. The blood glucose decremental $AUC_{(0-7)}$ and the maximum decrease in the blood glucose concentration were decreased by 44% and 36%, respectively, by rifampicin. Rifampicin decreased the AUC of glipizide by 22% and shortened its $t_{1/2}$ from 3.0 to 1.9 hours. No statistically significant differences in the blood glucose concentrations. Rifampicin moderately decreased the plasma concentrations and effects of glyburide but had only a slight effect on glipizide. The mechanism underlying the interaction between rifampicin and glyburide is probably induction of either CYP2C9 or P-glycoprotein or both. Induction of CYP2C9 would explain the increased systemic elimination of glipizide. It is probable that the blood glucose-lowering effect of glyburide is reduced during concomitant treatment with rifampicin (Niemi *et al.*, 2001).

6.6 Narcotic Analgesics

Methadone

Garfield *et al.* (1975) reported the occurrence of narcotic withdrawal symptoms in a group of patients on a methadone maintenance regimen who were also given rifampicin for coexistent tuberculosis. The serum concentrations of methadone were significantly lower than those seen when rifampicin was stopped.

Morphine

The potent analgesic morphine is metabolized by more than one UDP-glucuronosyltransferases (UGT_s) to the active metabolite morphine-6-

glucuronide and to morphine-3-glucuronide, which is devoid of analgesic activity. Fromm *et al.* (1997) investigated the influence of the potent enzyme inducer rifampicin on analgesic effects and pharmacokinetics of morphine, which is primarily, eliminated by phase 2 metabolism. The results showed that, the AUC of morphine and the C_{\max} were considerably reduced during coadministration of rifampicin. Since urinary recoveries of both morphine-3-glucuronide and morphine-6-glucuronide were also reduced during administration of rifampicin, there is no evidence for a contribution of UGI induction to the observed interaction.

6.7 Immunosuppressants

Cyclosporin

Hebert *et al.* (1992) showed that rifampicin not only induces the hepatic metabolism of cyclosporin but also decreases its bioavailability to a greater extent than would be predicted by the increased metabolism. The decreased bioavailability most probably can be explained by an induction of intestinal cytochrome P450 enzymes, which appears to be markedly greater than the induction of hepatic metabolism.

Tacrolimus

Tacrolimus is subject to extensive metabolism by CYP3A4 and is a substrate for P-glycoprotein-mediated transport. Tacrolimus was evaluated in six healthy male volunteers. Coadministration of rifampicin significantly increased tacrolimus clearance and decreased tacrolimus bioavailability. Rifampicin appears to induce both intestinal and hepatic metabolism of tacrolimus, most likely through induction of CYP3A4 and P-glycoprotein in the liver and small bowel (Hebert, *et al.*, 1999; Chenhsu *et al.*, 2000).

6.8 Antifungal Agents

Ketoconazole

Doble *et al.* (1988) observed the peak plasma ketoconazole levels and the AUC for ketoconazole were significantly diminished when taken in conjunction with rifampicin.

Itraconazole

Jaruratanasirikul and Sriwiriyan, (1998) have found concentration itraconazole were higher when it was administered alone than when it was administered with rifampicin. Coadministration of rifampicin results in undetectable levels of itraconazole in all subjects except one normal volunteer. The mean AUC_{0-24} was 0.39 vs 3.28 $\mu\text{g/ml.hr}$ with and without rifampicin respectively, in healthy normal volunteers. Therefore, the estimated minimum decrease of the mean AUC_{0-24} of itraconazole when administered with rifampicin was approximately 88% compared with itraconazole was administered alone. Rifampicin has a very strong inducing effect on the metabolism of itraconazole, so that these two drugs should not be administered concomitantly.

6.9 Antituberculosis Drugs

Rifampicin has no effect on either the metabolism or excretion of pyrazinamide. The pharmacokinetic properties of both drugs were not altered significantly when rifampicin and isoniazid were administered in conjunction. Although some clinical studies and case reports suggest that the combination of rifampicin and isoniazid may be more hepatotoxic than either drug alone, Holdiness (1984) has emphatically reported that the vast majority of individuals receiving both drugs together do not develop clinically evident

synergistic hepatotoxicity.

6.10 Drug for Human Immunodeficiency Virus (HIV) Infection

Zidovudine

14 days of coadministration with rifampicin significantly increased zidovudine oral clearance (89%) and formation clearances to 5'-glucuronosyl zidovudine metabolite (100%) and 3'-amino metabolite (82%). Correspondingly, there were decreases in maximum plasma concentration (43%), AUC (47%) and urine recovery (37%) of zidovudine. After stopping rifampicin for 14 days, values of these pharmacokinetic parameters returned to within 26% of baseline. Rifampicin induced zidovudine glucuronidation and amination pathways resulting in decreased plasma and urine exposures to zidovudine. The magnitude of the residual inductive effect was minimal at 14 days after stopping rifampicin (Gallicano *et al.*, 1999; Burger *et al.*, 1993).

Protease Inhibitors

Saquinavir is extensively metabolized by cytochrome P-450 enzymes, primarily CYP3A4. In a premarketing clinical study of 12 healthy volunteers, a dose of saquinavir, 600 mg, was given 3 times daily concurrently with rifampicin, 600 mg/d. At steady state concentrations, a dramatic decrease of about 80% in both AUC and C_{\max} of saquinavir was observed. Based on the significance of this study, concomitant administration of saquinavir with rifampicin should be avoided. Studies of this interaction with a new formulation of saquinavir, which has enhanced bioavailability, are being conducted (Strayhorn *et al.*, 1997).

Since ritonavir and indinavir are both primarily metabolized by

CYP3A4, induction of metabolism by rifampicin is probable. Healthy volunteers were given either concomitant rifampicin, 600 mg/d, and indinavir, 800 mg every 8 hours, or indinavir alone. Rifampicin use resulted in a drastic 92% decrease in indinavir AUC. Rifampicin induction of ritonavir metabolism appears to be less significant. Administration of ritonavir, 500 mg twice daily, with rifampicin 600 or 300 mg/d, resulted in a decrease in ritonavir AUC by 35% and C_{max} by 25%. A newer protease inhibitor, nelfinavir, is also primarily metabolized by CYP3A4. Rifampicin administration has resulted in a 3- to 11-fold increase in nelfinavir oral clearance in 12 healthy volunteers (Strayhorn, 1997; Moreno *et al.*, 2001).

The coadministration of amprenavir and rifampicin resulted in significant changes in the pharmacokinetics of amprenavir, including an 82% decrease in the AUC_{ss} and an increase of greater than 5-fold in amprenavir CL/F . This most likely reflects induction of hepatic and intestinal CYP3A4 by rifampicin, and possibly enhancement of P-glycoprotein transport, resulting in an increase in clearance of amprenavir (Polk *et al.*, 2001).

Delavirdine

Borin *et al.* (1997) studied the effect of rifampicin on delavirdine pharmacokinetics. Twelve patients received delavirdine, 400 mg every 8 hours for 30 days ; 7 of the patients also received rifampicin , 600 mg/d, on days 16 to 30 of delavirdine therapy. In the rifampicin group, oral clearance of delavirdine increased nearly 27-fold, and plasma through concentrations of delavirdine were almost negligible after 2 weeks of concomitant therapy. Based on the results of this study, concurrent administration of delavirdine with rifampicin should be avoided.

6.11 Psychotropic Agents

Benzodiazepines

Backman *et al.* (1996) studied 10 patients receiving rifampicin, 600 mg/d, or a placebo for 5 days. Midazolam, 15 mg/d orally, was introduced on day 6. Midazolam values for C_{\max} decreased by 94%, and for AUC by 96%; the elimination half-life was 40% compared with the control value. The mechanism of this interaction is postulated to be due to enzymatic induction in the gut wall, since AUC decreased much more than the half-life.

Rifampicin also has a marked pharmacokinetic and pharmacodynamic effect on triazolam. In a double-blind, randomized, cross-over study of 10 healthy volunteers, use of rifampicin, 600 mg/d, for 5 days reduced triazolam AUC by 95% and C_{\max} by 88%. Consistent with the drastic reduction in serum concentrations, the patients experienced virtually no pharmacologic effect from the single 0.5-mg oral dose of triazolam as tested by psychomotor performance (Villikka *et al.*, 1997a).

Zolpidem

5-day pretreatment with rifampicin caused a great reduction in the plasma concentrations and effects of zolpidem. After rifampicin, the total AUC of zolpidem averaged only 27%, the C_{\max} of zolpidem was decreased by 58% and the $t_{1/2}$ was shortened from 2.5 ± 0.2 to 1.6 ± 0.1 hours by rifampicin. The effects of zolpidem are considerably reduced by rifampicin because of enhanced metabolism of zolpidem (Villikka *et al.*, 1997b).

6.12 Antimalarial Drugs

Quinine

Wanwimolruk *et al.* (1995) studied the effect of rifampicin

pretreatment on the pharmacokinetics of quinine after a single oral dose (600 mg quinine sulphate) was studied in nine healthy young Thai male volunteers. The mean clearance (Cl/F) of quinine coadministered with rifampicin was significantly greater than that of quinine alone. The mean difference in clearance from the control treatment was 0.73 l/h/kg, with 95% confidence interval (C.I.) of 0.48 to 0.98. The unbound clearance (Cl_u/F) of quinine, which reflects the activity of the drug metabolizing enzymes was considerably greater (6.9-fold) in subjects when rifampicin was administered with quinine than that of quinine alone. The mean elimination half-life of quinine when coadministered with rifampicin was significantly shorter than when quinine was given alone. These results indicate that rifampicin pretreatment caused a marked increase (6.2-fold) in the clearance of quinine, possibly due to enzyme induction. The extent to which the elimination of quinine is enhanced by the concomitant administration of rifampicin is likely to have important clinical consequences. Although the clinical significance of these finding is unknown, they indicate the need for caution in the administration of quinine to patients who are concurrently taking rifampicin as an anti-tuberculosis medication.

Mefloquine

Rititid *et al.* (2000) indicated that rifampicin significantly decreased the AUC of mefloquine by 68%. C_{max} by 19%, and elimination $t_{1/2}$ by 63%, whereas the t_{max} of mefloquine was unaffected. The Cl/f of mefloquine was significantly increased by 281%. After administration of rifampicin, the C_{max} of the carboxylic acid metabolite of mefloquine was significantly increased by 47%, whereas the $t_{1/2}$ was significantly decreased by 39% and t_{max} by 76%. The AUC and Cl of the mefloquine metabolite were increased by 30% and 25%,

respectively, but were not significantly different from the control phase. The results indicated that rifampicin reduces the plasma concentration of a single oral dose of 500 mg mefloquine by increasing metabolism of mefloquine in the liver and gut wall. The CYP3A4 isozyme most likely plays an important role in the enhanced metabolism of mefloquine. Simultaneous use of rifampicin and mefloquine should be avoided to optimize the therapeutic efficacy of mefloquine and prevent the risk *Plasmodium falciparum* resistance in malarial treatment.

6.13 Others

Chloramphenicol

Prober (1985) reported decrease of 86.5 and 63.8% in serum concentrations of chloramphenicol in 2 children treated with intravenous chloramphenicol and rifampicin concomitantly. Kelly et al. (1988) also found chloramphenicol-rifampicin interactions in children. Although additional studies are required to validate this interaction, serum concentrations of chloramphenicol may be tested as a precautionary measure when this drug is administered concomitantly with rifampicin.

Theophylline

Gillum *et al.* (1996) observed that rifampicin treatment, 300 mg/day for 14 days, results in a significant increase in clearance of theophylline and the mean AUC for theophylline decreased by 27%.

Ondansetron

The mean total AUC of orally administered ondansetron after rifampicin pretreatment was reduced by about 50% and the elimination $t_{1/2}$ by 38% the bioavailability of oral ondansetron was reduced from 60% to 40% by

rifampicin. The Cl of intravenous ondansetron was increased 83% by rifampicin. Rifampicin reduced the $t_{1/2}$ of intravenously administered ondansetron by 46% and AUC by 48%. Rifampicin considerably decreased the plasma concentrations of ondansetron after both oral and intravenous administration. The interaction is most likely the result of induction of the CYP3A4-mediated metabolism of ondansetron (Villikka *et al.*, 1999).

Trimethoprim and Sulfamethoxazole

Statistically significant, 47 and 23% decreases in trimethoprim and sulfamethoxazole mean $AUC_{(0-24)}$, respectively, were observed after administration of rifampicin. *N*-Acetyl- sulfamethoxazole profiles without and with rifampicin were similar. The steady-state $AUC_{(0-24)}$ metabolite/parent drug ratio increased by 32% with rifampicin administration. The results show that rifampicin reduces profiles of trimethoprim and sulfamethoxazole in serum of HIV-infected patients (Ribera *et al.*, 2001).

Lamotrigine

Rifampicin was able to reduce the AUC of lamotrigine representing a measure of the total body load of drug, and to increase both the Cl/F of lamotrigine and the amount of lamotrigine in urine excreted as glucuronide. Additionally, coadministration of rifampicin was associated with a 30% shortening of the lamotrigine half-life. Rifampicin altered pharmacokinetics of lamotrigine due to induction of the hepatic enzymes responsible for glucuronidation (Ebert *et al.*, 2000).

Simvastatin

Rifampicin reduced the mean AUC of simvastatin and simvastatin acid by 87% and 93%, respectively. The C_{max} values of both simvastatin and

simvastatin acid were decreased by 90% by rifampicin. Rifampicin had no significant effect on the t_{\max} or $t_{1/2}$ of simvastatin or simvastatin acid. Rifampicin greatly decreased the plasma concentrations of both simvastatin and simvastatin acid. Because the elimination half-life of simvastatin was not affected by rifampicin, induction of the CYP3A4-mediated first-pass metabolism of simvastatin in the intestinal and the liver probably explains this interaction (Kyrklund *et al.*, 2000).

Fexofenadine

Hamman *et al.* (2001) showed that a significant increase in the oral clearance of fexofenadine after rifampicin treatment. The C_{\max} of fexofenadine was also significantly reduced by rifampicin treatment, t_{\max} and fraction unbound of fexofenadine showed no significant difference between control and treatment. The amount of azacyclonol, a CYP3A4 mediated metabolite of fexofenadine, eliminated renally increased on average 2-fold after rifampicin dosing; however, this pathway accounted for less than 0.5% of the dose. This study showed that rifampicin effectively increased fexofenadine oral clearance. They concluded that the cause of the increased oral clearance of fexofenadine is a reduced bioavailability caused by induction of intestinal P-glycoprotein.

Cytochrome P-450

1. Introduction

In human, the metabolizing processes of foreign compounds require a large number of enzymes and almost all occurs in the liver. Most of the enzymes have been classified as belonging to phase I or phase II pathways of metabolism. Phase I enzymes include reductase, oxidase and hydrolases. Phase II enzymes are all transferases. These reactions will transform a hydrophobic compound into a form that is more water-soluble and can be easily eliminated from the organism through urine or bile.

Among drug-metabolizing enzymes in phase I pathway, cytochrome P450 (P450s or CYPs) are the most active one. These enzymes are also principally responsible for activation of procarcinogens and promutagens. Most clinically used drugs are metabolized to some degree by P450s.

P450s represent a superfamily of enzymes. They are found in animals, plants, yeast and bacteria. In mammals, some P450s are involved in pathways of steroid biosynthesis and do not metabolize foreign compounds. However, the vast majority of these enzymes, the foreign compound metabolizing P450s, appear to oxidize chemicals that are not normal constituents of the body. P450s are named with the root CYP followed by an Arabic number and upper case letter designating the family and subfamily, respectively. Individual P450 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 oxidoreductase, 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 P450-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 P450s 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 P450 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 P450 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

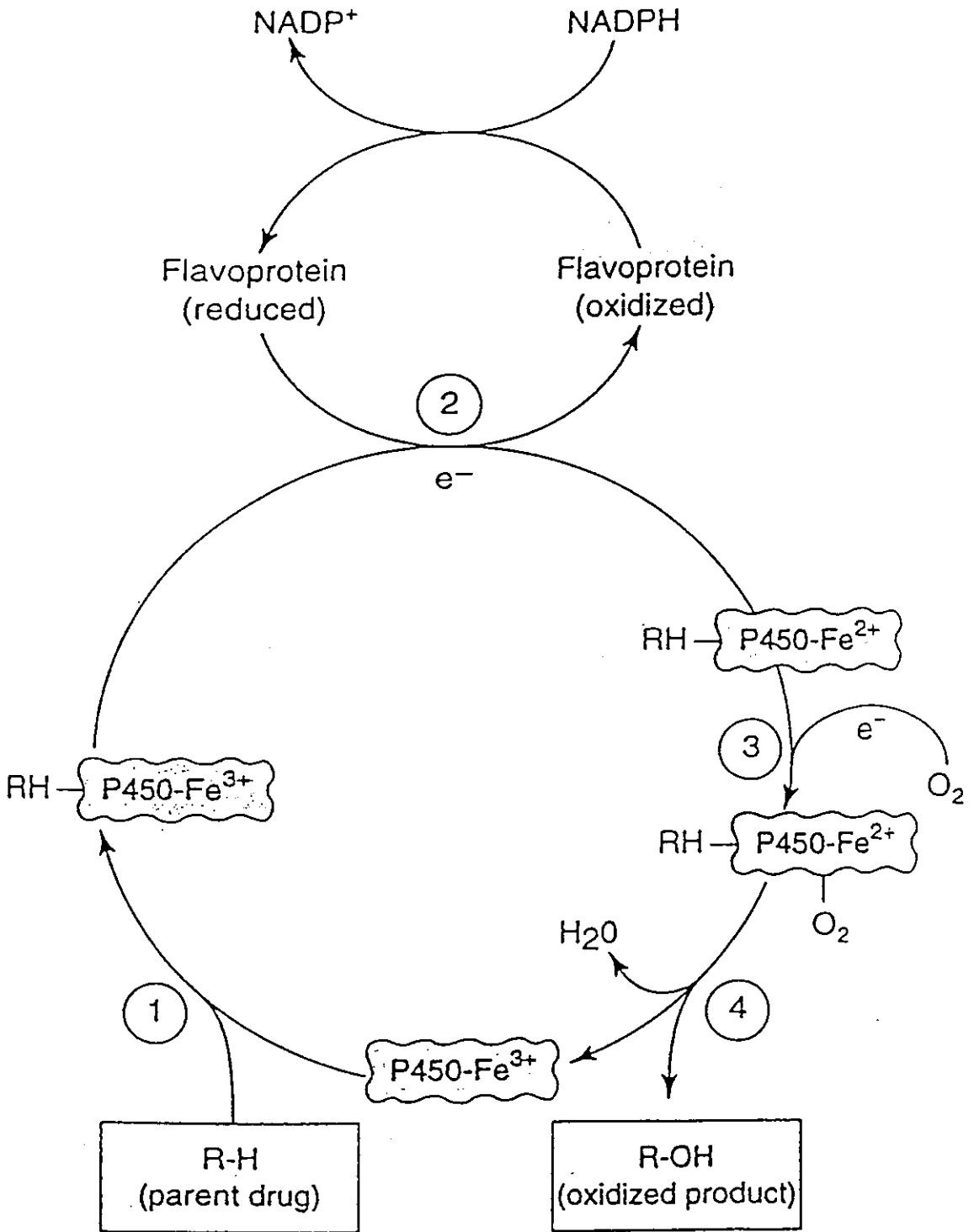


Figure 5 Cytochrome P-450 cycle in drug oxidations

(Correia, 1998 :52)

(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 P450, 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 P450 generally lacks absolute complementarity, substrates often bind to the enzyme active site in several different configurations, resulting in multiple metabolites with regio- and stereospecificity unique to each isoform.

3. Mechanisms of Inhibition of P450

The catalytic cycle of P450 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 P450 reductase
- (iii) binding of molecular oxygen
- (iv) transfer of a second electron from P450 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 P450 enzyme activity, step(i), (iii) and (vi) are particularly vulnerable to inhibition.

The mechanisms of P450 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 P450 inhibitors are nitrogen containing drug, including imidazole, 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 P450 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 P450, an apparent result of an intrinsic low binding affinity to microsomal P450. This latter property is most probably because of the low lipophilicity of cimetidine ($\log P=0.4$). On the other hand, ketoconazole, a potent P450 inhibitor, has a high lipophilicity ($\log P=3.7$). Similarly, fluconazole contains a triazole that binds to the prosthetic haem iron but is a weak reversible P450 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 P450 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 P450 (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 P450 enzymes to form inhibitory metabolites. These metabolites can form stable complexes with the prosthetic haem of P450, called metabolic intermediate (MI) complex, so that the P450 is sequestered in a functionally inactive state. MI complexation can be reversed, and the catalytic function of ferric P450 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 P450 functional activity. However, in *in vivo* situations, the MI complex is so stable that the P450 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 P450

Drug containing certain functional group can be oxidized by P450 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 P450 may result from irreversible alteration of haem or protein, or a combination of both. In general, modification of the haem group invariably inactivates the P450, 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 P450

One of the intriguing aspects of the P450 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, P450 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 P450 induction are often overlooked in clinical studies.

Although the phenomenon of P450 induction has been known for more than 4 decades, we have just begun to uncover the mechanisms involved in induction only in recent years. 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 P450 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 P450 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 P450 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 P450

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 H₁ 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 P450 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 (Lin and Lu, 1998).

5.2 Induction of P450

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 P450 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 progestogenic 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 might be a toxicity of excessive accumulation of drug when the inducer is withdrawn and enzyme activity returns to normal (Lin and Lu, 1998).

Table 1. Major human liver cytochrome P450 (CYP) enzymes (Lin and Lu, 1998)

CYP	Drug substrate	Marker substrate/reaction	Inhibitor	Inducer
1A2	paracetamol, caffeine, ondansetron, phenacetin, tacrine, tamoxifen, theophylline	phenacetin/O-de-ethylation	furafylline	smoking, charred food
2A6	coumarin, nicotine	coumarin/7-hydroxylation	dithiocarb sodium (diethyldithiocarbamate)	
2C9	diclofenac, flurbiprofen, losartan, phenytoin, piroxicam, tienilic acid, tolbutamide, torasemide, (S)-warfarin	tolbutamide/ methyl hydroxylation	sulfaphenazole	barbiturates, rifampicin
2C19	diazepam, (S)-mephenytoin, omeprazole, pentamidine, propranolol, (R)-warfarin	(S)-mephenytoin/ 4-hydroxylation		
2D6	bufuralol, codeine, debrisoquine, desipramine, encainide, dextromethorphan, fluoxetine, haloperidol, imipramine, nortriptyline, paroxetine, propafenone, propranolol, sparteine	bufuralol/1-hydroxylation	quinidine, ajmaline	
2E1	paracetamol, caffeine, chlorzoxazone, enflurane, theophylline	chlorzoxazone/6-hydroxylation	dithiocarb sodium	alcohol, isoniazid
3A4	benzphetamine, clarithromycin, codeine, cyclosporin, dapson, diazepam, erythromycin, felodipine, tacrolimus, indinavir, lovastatin, midazolam, nifedipine, carbamazepine, losartan, quinidine, taxol, terfenadine, verapamil	testosterone/6 β -hydroxylation	gestodene, troleandomycin, L-754,394, ketoconazole, itraconazole	barbiturates, rifampicin, dexamethasone, carbamazepine