

CHAPTER 2

LITERATURE REVIEW

Diethylcarbamazine

Diethylcarbamazine (DEC) [1-diethylcarbamyl 4-methyl piperazine] is the most important compound for the treatment of filarial infections (Gelband, 1994). It is a first-line agent for control and treatment of lymphatic filariasis and for therapy of tropical pulmonary eosinophilia caused by *Wuchereria bancrofti* and *Brugia malayi* (Ottensen and Ramachandran, 1995). The most promising group of antifilarial compounds were piperazine derivatives, of which DEC is the most important (Hawking, 1979; Mackenzie and Kron, 1985). DEC has the chemical structure as shown in Figure 1.

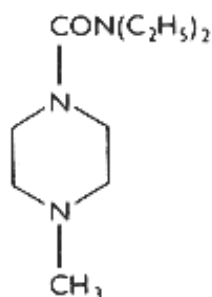


Figure 1 Structure of diethylcarbamazine

Chemical and Physical Properties

Chemical structure : $C_{10}H_{21}N_3O$, $C_6H_8O_7$

Molecular weight : 391.4

(Reynolds, 1993)

pK_a : 7.37

(Lee *et al.*, 1997)

Solubility :

in water : freely soluble

in alcohol : 1 in 35 of alcohol

in acetone, chloroform, and ether : insoluble

(Reynolds, 1993)

1. Pharmacodynamic Properties

1.1 Mechanism of Action

Microfilarial form of susceptible filarial species are most affected by DEC, which elicits rapid disappearance of these developmental forms of *W. bancrofti*, *B. malayi*, and *Loa Loa* from human blood. The drug causes microfilariae of *Onchocerca volvulus* to disappear from skin but does not kill microfilariae in nodules that contain the adult (female) worms. It does not affect the microfilariae of *W. bancrofti* in a hydrocele, despite penetration into the fluid. The drug is inactive *in vitro* against microfilariae. However, *in vivo* it produces rapid destruction of the microfilariae of *W. bancrofti*, *B. malayi*, and *B. timori* (the 3 lymphatic-dwelling filarial infecting humans) (Mandell *et al.*, 1990; AMA, 1991; Hawking, 1979). It is also active *in vivo* against *O. volvulus*, *L. loa*, and to a lesser extent against *Mansonella perstans* (Mandell *et al.*, 1990; AMA, 1991). There is evidence that DEC kills worms of adult *L. loa* and probably adult *W. bancrofti* and *B. malayi* as well.

The mechanism of action of DEC on susceptible microfilariae is not well understood (Martin *et al.*, 1997; de Silva *et al.*, 1997). DEC may perturb arachidonic acid metabolism in both microfilariae and host endothelial cells with resulting vasoconstriction and aggregation of host platelets and granulocytes around membrane damaged parasites (Tracy and Webster, 2001). The mechanism of filaricidal action of DEC against adult worms is unknown (Hawking, 1979). Some studies suggested that DEC compromises intracellular processing and transport of certain macromolecules to the plasma-membrane (Spiro *et al.*, 1986). The drug also may affect specific immune and inflammatory responses of the host by as yet undefined mechanisms (Mackenzie and Kron, 1985; Martin *et al.*, 1997).

1.2 Immunoglobulin responses

Terhell *et al.* (2003) indicated that level of anti-filarial IgG4 in that population was reduced considerably following therapy with DEC. There was a significant correlation between percentage reduction in specific IgG4 and the number of days DEC was taken under observation (n=60; $P=0.001$). The post-treatment reduction in specific IgG4 in study participants with 10-19, 20-29 and 30-40 treatment days were observed. Individuals with 30-40 treatment days (total DEC dosage of at least 180 mg/kg) showed a higher overall reduction in specific IgG4 levels than persons with 10-19 or 20-29 treatment days.

2. Pharmacokinetic Properties

2.1 Absorption

DEC is absorbed rapidly from the gastrointestinal tract. Following a single radioactively labeled dose of DEC citrate ($[^{14}\text{C}]$ DEC) 0.5 mg per kg body-weight, administered by mouth as an aqueous solution, to 6 patients with onchocerciasis, peak plasma concentration of 100 to 150 ng/ml were achieved within 1 to 2 hours, followed by a sharp decline, then a marked secondary rise 3 to 6 h after dosing followed by a steady decline. In 5 healthy volunteers receiving a 50 mg tablet dose of DEC citrate, absorption was rapid, with peak plasma concentrations similar to the above study being attained within 2 h (Edwards *et al.*, 1981). Whereas administrations with 150 mg and 6 mg/kg body-weight were produced mean peak plasma concentration at 500 to 637 ng/ml in 2-3 h (Bolla *et al.*, 2002) and 1254 to 2348 ng/ml in 2-4 h (Shenoy *et al.*, 2000), respectively. Since no parenteral formulation DEC exists, the absolute bioavailability of the drug is unknown.

2.2 Distribution

In animal, DEC distributes in similar amounts to most tissues, except adipose tissue. High drug concentrations have been found in the pituitary gland, lymph nodes, adrenal medulla, and salivary gland. DEC crosses the blood-brain barrier (Mandell *et al.*, 1990; Hawking, 1979). Volume of distribution (V_d/F) was estimated in 5 healthy volunteers to be 240 ± 104 L (range, 107 to 371 L). In 6 patients with onchocerciasis mean V_d was 201 ± 48 L (range, 143 to 271 L), indicating significant distribution outside total body water spaces and into less well-perfused tissue. DEC is not significantly bound to plasma protein (Bolla *et al.*, 2002). There are no reports of the transfer of DEC into breast milk (Edward *et al.*, 1988).

2.3 Elimination

DEC is rapidly and extensively metabolized by liver (AMA, 1991; Gilman *et al.*, 1990; Edward *et al.*, 1981; Hawking, 1979). In patients with onchocerciasis, systemic clearance of DEC (Cl/F) was 11.6 ± 2.3 L/h (range, 9.0 to 15.7 L/h). Somewhat greater values (mean \pm SD. 18.8 ± 9.4 L/h; range, 7.4 to 29.5 L/h) were found in healthy volunteers. Values for the apparent terminal phase elimination half-life ranged from 9 to 13 h (mean 12 ± 1.5 h) in patients with onchocerciasis and 5 to 13 h (mean \pm SD. 10 ± 3 h) in healthy subjects (Edwards *et al.*, 1981). The half-life is prolonged with the presence of an alkaline urine or renal impairment. The half-life was 2 to 9 h when urine was acidified (pH less than 5.5) with ammonium chloride and prolonged

to 6-17 h with urinary alkalization (pH greater than 7.5) by sodium bicarbonate (Adjepon-Yamoah *et al.*, 1982; Edward *et al.*, 1981).

Elimination of DEC in both patients and healthy subjects is by renal and extrarenal routes. $51.8 \pm 9.0\%$ of the administered dose was excreted unchanged in the urine of onchocerciasis patients, and $48.9 \pm 10.7\%$ excreted in the urine of healthy volunteers. Less than 10% was excreted as DEC-N-oxide; between 4 to 5% was recovered in the faeces (Edward *et al.*, 1981).

Adjepon-Yamoah *et al.* (1982) studied DEC-administration in renal failure. Results in patients with chronic renal impairment and healthy subjects, given a single 50 mg dose of DEC by mouth, indicated that the plasma half-life of DEC prolonged and its 24-h urinary excretion considerably reduced in those with moderate and severe degrees of renal failure. Mean plasma half-lives in 7 patients with severe renal failure (creatinine clearance less than 25 ml per minute), 5 patients with moderate renal failure (creatinine clearance between 25 and 60 ml per minute), and 4 healthy subjects were 15.1, 7.7, and 2.7, respectively. The patient with the longest plasma half-life of 32 h did not have the poorest renal function, but it was considered likely that the abnormally slow elimination of DEC was due to the high urine pH (7) resulting from sodium bicarbonate therapy. A further patient with a half-life longer than expected also had less acidic urine.

Urinary excretion of DEC is pH-dependent. In healthy volunteers receiving a single oral dose of DEC citrate 50 mg, total urinary excretion and renal clearance of DEC were significantly reduced when an alkaline, as opposed to acidic, urinary pH was maintained. Additionally, the terminal elimination half-life of DEC and the area under the plasma concentration-time curve ($AUC_{0 \rightarrow \infty}$) were significantly greater at alkaline pH than under acidic conditions. The proportion of DEC excreted as the N-oxide metabolite was not affected by urinary pH (Edwards *et al.*, 1981).

Awadzi *et al.* (1986) studied the effect of moderate urine alkalisation on low dose DEC therapy in patients with onchocerciasis. 21 patients with moderate to heavy infections with *O. volvulus* were treated with 25 mg of DEC twice daily for 10 days. In 11 patients, the urine was made alkaline with sodium bicarbonate, 2 g administered 6 hourly for three doses daily beginning 1 day before DEC was started and continued throughout the DEC therapy. Ten patients served as control. The mean plasma concentration vs. time values for DEC between control and

bicarbonate group in day 2, 3, 5, and 10 are 52.2 ± 28.8 vs. 78.8 ± 38.7 ng/ml, 63.8 ± 39.0 vs. 117.1 ± 55.4 ng/ml, 73.1 ± 40.1 vs. 146.1 ± 82.2 ng/ml and 57.6 ± 18.6 vs. 137.4 ± 101.5 ng/ml, respectively. The values obtained in the control group were significantly lower than those found in bicarbonate group. The AUC values in control group (2868 ± 2051 ng.h/ml) and in bicarbonate group (3318 ± 1189 ng.h/ml) were not significantly different. The plasma elimination half-life of DEC was significantly increased from 9.3 ± 2.1 h (control group) to 11.2 ± 3.1 h (bicarbonate group). The total urinary excretion of DEC was significantly less in patients receiving sodium bicarbonate (31.9 ± 13.6 mg) than in control (49.9 ± 17.8 mg) ($P > 0.01$).

3. Adverse effects

Adverse effects directly attributable to DEC, which are generally mild and transient (disappear within a few days despite continuation of therapy). These reaction include anorexia, nausea, headache, and at high doses, vomiting (Tracy and Webster, 2001). Major adverse effects also occur as a result of the release of proteins from dying microfilariae or adult worms (Rosenthal *et al.*, 1998).

Reactions occurring during DEC treatment of lymphatic filariasis are basically of 2 types: pharmacological dose-dependent responses and a response of the infected host to the destruction and death of parasites. Reactions of the first type include weakness, dizziness, lethargy, anorexia, and nausea. They begin within 1 to 2 h of taking DEC and persist for few hours. Reactions of the second type are less likely to occur and less severe in bancroftian than in brugian filariasis. They may be systemic or local both with and without fever. Systemic reactions may occur few hours after the first oral dose of DEC and generally do not last for more than 3 days. They include headache, aches in other parts of the body, joint pain, dizziness, anorexia, malaise, transient haematuria, allergic reaction, vomiting, and sometimes attacks of bronchial asthma in asthmatic patients. Fever and systemic reactions are positively associated with microfilaraemia. Systemic reactions are reduced if DEC is given in divided doses or in repeated small doses. They eventually cease spontaneously and interruption of treatment is rarely necessary; symptomatic treatment with antipyretics or analgesics may be helpful. Local reactions tend to occur latter in the course of treatment and last longer; they also disappear spontaneously and interruption of treatment is not necessary. Local reactions include lymphadenitis, abscess,

ulceration, and transient lymphoedema; funiculitis and epididymitis may also occur in bancroftian filariasis (Renolds, 1993).

Reactions are especially severe in patients heavily infected with *O. volvulus*. They usually are less serious in *B. malayi* or *L. loa* infections and usually are mild in bancroftian filariasis, but the drug occasionally induces retinal hemorrhages and severe encephalitis in patients heavily infected with *L. loa*. In patients with onchocerciasis, there usually is a typical reaction (termed a *Mazzotti reaction*) that occurs within a few hours after the first oral dose. This consists of intense itching and skin rashes, enlargement and tenderness of the lymph nodes, sometimes rash, fever, tachycardia, arthralgia, and headache. These symptoms persist for 3 to 7 days and then subside, after which quite high doses can be tolerated. Ocular complications include limbitis, punctuate keratitis, uveitis, and atrophy of the retinal pigment epithelium. In patients with bancroftian or brugian filariasis, nodular swellings may occur along the course of the lymphatics, and there is often an accompanying lymphadenitis. This reaction also subsides within a few days. Leukocytosis is common. Eosinophilia may increase with treatment. Proteinuria may also occur. DEC appears to be safe for use during pregnancy.

4. Precautions and Contraindications

Population-based chemotherapy with DEC should be avoided in areas where onchocerciasis or loiasis is endemic, even though the drug can be used to protect foreign travelers from these infections. Pretreatment with glucocorticoids and antihistamines often is undertaken to minimize indirect reactions to DEC that result from dying microfilariae. Dosage reduction must be considered for patients with impaired renal function or persistent alkaline urine (Tracy and Webster, 2001).

5. Therapeutic Uses

Dosages of DEC used to prevent or treat filarial infections have evolved empirically and vary according to local experience. Recommended regimens differ according to whether the drug is used for population-based chemotherapy, control of filarial disease, or prophylaxis against infection.

W. bancrofti, B. malayi, and B. timori

For mass treatment with the objective of reducing microfilaremia to sub-infective levels for mosquitoes, the recent introduction of DEC into table salt (0.2% to 0.4% by weight of the base) has markedly reduced the prevalence, severity, and transmission of lymphatic filariasis in many endemic areas. Moreover, single 6 mg/kg doses given orally every 6 to 12 months have proven just as effective as former prolonged daily dosage regimens. A major discovery was that DEC given annually as a single oral dose of 6 mg/kg was most effective in reducing microfilaremia when given along with a single dose of another antifilarial agent. Initial studies were done with ivermectin as the partner drug, but more recent evidence indicates that 400 mg of albendazole taken orally in addition to DEC is an even more appropriate choice for mass chemotherapy. Adverse reactions to microfilarial destruction, greater after the oral DEC tablet, usually are well tolerated. However, mass chemotherapy with DEC should not be used in regions where onchocerciasis or loiasis coexist because, even in the table salt formulation, this drug may induce especially severe reactions related to parasite burden in these infections (Tracy and Webster, 2001).

DEC, given in doses of 2 mg/kg three times daily for 14 days, causes rapid disappearance of symptoms of tropical eosinophilia, the pulmonary inflammatory response typical of infection with *W. bancrofti* or *B. malayi*. After taking a small test dose of 50 mg, asymptomatic individuals with microfilaremia should be treated with “standard” courses DEC, *e.g.*, 6 mg/kg in 3 divided doses per day for 14 to 21 days; the 21 day period is customary for *B. malayi*. Such therapy should minimize further lymphatic and renal damage. It is largely ineffective against more advanced complications of lymphatic filariasis, such as lymphagitis and chronic obstructive lymphedema (*elephantiasis*), which respond better to antibiotics and maintenance of good local hygiene, respectively. A monthly dose of 50 mg of DEC is effective for prophylaxis against lymphatic filariasis (Tracy and Webster, 2001).

When DEC is used for the treatment of lymphatic filariasis, the microfilariae rapidly disappear. Some of the chronic clinical manifestations of the disease may improve, although repeated courses may be necessary; large hydroceles or elephantiasis with deformities tend not to show any improvement. Tropical pulmonary eosinophilia responds but about 20% of patients may relapse and require retreatment. WHO recommends for bancroftian filariasis a dose of 6 mg/kg body-weight daily in divided doses after food for 12 consecutive days. As the

incidence of adverse effects is greater when treating malayan filariasis with DEC, doses tend to be lower and range from 3 to 6 mg/kg daily for 6 to 12 days. Repeated courses may be necessary. The manufacturers recommended a treatment period of 3 weeks for both types of infection (Reynolds, 1993).

O. volvulus* and *L. loa

DEC is no longer recommended for initial treatment of onchocerciasis, because it causes severe reactions related to microfilarial destruction. Such reactions are far less severe in response to ivermectin, the drug now preferred for this infection. DEC, despite its drawbacks, remains the best available drug for therapy of loiasis. Treatment is initiated with test doses of 1 mg/kg daily for 2 to 3 days, escalating to maximally tolerate daily doses of 8 to 10 mg/kg for a total of 2 to 3 weeks. Low test doses are used, often accompanied by pretreatment with glucocorticoids or antihistamines, to minimize reactions to dying microfilariae and adult worms; these reactions consist of severe allergic reactions and, occasionally, meningoencephalitis and coma from invasion of the CNS by microfilariae. Repeated courses of treatment with DEC, separated by 3 to 4 weeks may be required to cure loiasis, and doses of 300 mg weekly have been proven to be effective for prophylaxis against this infection. Ivermectin does not provide a good alternative to DEC for treatment of loiasis, but albendazole may prove to be useful in patients who either fail therapy with DEC or who cannot take the drug (Klion *et al.*, 1999).

DEC is clinically effective against microfilariae and adult worms of *Dipetalonema streptocerca*, but filariasis due to *Mansonella perstans*, *M. ozzardi*, or *Dirofilaria immitis* responds minimally to this agent. Although therapy with DEC, 6 mg/kg daily in divided doses for 7 to 10 days, has been recommended for treatment of toxocariasis, the drug is considered experimental for this indication (Tracy and Webster, 2001).

6. Analytical review

DEC was determined in human urine by $^1\text{H-NMR}$ spectroscopy. Urine sample was mixed with 10% of deuterium oxide (D_2O) as a spectrometer field frequency lock, which is the sample preanalyzed required. Tailored excitation with the 1331 pulse was used for which the T_1 relaxation time was 1 s. Determination of DEC used the standard addition method. These results showed a good accuracy and precision. As little as about 5 $\mu\text{g/ml}$ of DEC could be observed in urine. Analysis of urine from volunteers receiving a single therapeutic dose of DEC

(6mg/mkg body weight orally) showed that the drug was eliminated in unchanged form during 2 days. This method can detect DEC-*N*-oxide. Although, ¹H-NMR spectroscopy is convenient method, but limit of detection (LOD) is 5 µg/ml. It is higher than other methods, which are gas chromatography (GC) and high performance liquid chromatography (HPLC), can detect about 20 and 70 ng/ml, respectively (Jarazewski *et al.*, 1996).

Competitive enzyme-linked immunosorbent assay (ELISA) was developed for the determination of the concentration of DEC in biological fluid. Since DEC has no functional group to conjugate with bovine serum albumin (BSA), *N*-(2-aminoethyl)-*N*-ethyl-1-piperazinecarboxamide (DEC-NH₂) was first synthesized. This compound was then converted to carboxyl DEC marker, carboxyl DEC (DEC-COOH), and conjugated to BSA and to poly-L-lysine for use as immunogen and solid-phase marker, respectively. The competitive ELISA was conducted by simultaneously incubating DEC with mouse anti-DEC antiserum over DEC-poly-L-lysine solid phase. Subsequently, the binding of anti-DEC antibody was detected by using sheep anti-mouse IgG peroxidase conjugate as a tracer. The cross-reactivity of DEC-metabolites and related compounds was less than 0.047% ivermectin did not react with anti-DEC antibodies even at a high concentration (100 µg/ml). Competitive ELISA standard curve for DEC was yield between 1 and 100 µg/ml. The recoveries of intra-day and inter-day were 99.25-108.60% and 98.14-102.04%, respectively (n=5). These results of %CV were satisfactory. DEC concentration was determined in 4 male jirds following a single intraperitoneal administration of DEC-citrate (100 mg/kg). The highest concentration (10 µg/ml) was recorded 10 minutes after the administration of DEC. Thereafter the level of DEC rapidly decreased to 0.18 µg/ml at 4 h. The detectable limit in their assay was 1 ng/ml in assay buffer (Mitsui *et al.*, 1996).

GC analysis of DEC in plasma can be complicated by the presence of the metabolite, since the thermally unstable DEC-*N*-oxide is converted to a material which co-elutes with DEC under the conditions of the analysis. This method separated DEC-*N*-oxide from DEC in plasma using solid-phase extraction with subsequent gas chromatographic analysis using a nitrogen specific detector. Physicochemical properties of the compounds of interest were characterized by differential scanning calorimetric thermal analysis and pK_a determinations. 1-Diethylcarbamy-4-ethylpiperazine (E-DEC) was the internal standard. The standard curve of DEC is linear in the range of 10 to 200 ng/ml. The LOD and limit of quantification (LOQ) were 4 and 10 ng/ml, respectively. Reproducibility at 10, 100 and 200 ng/ml concentration points of the

standard curve gave CV of 6.1%, 7.8% and 1.6%, respectively. Recovery solid-phase extraction is 99.3% for DEC and 94.8% for the internal standard. The determined pK_a values were 7.37 for DEC and 7.00 for DEC-*N*-oxide. Thermal analysis defined the thermal stability of DEC citrate DEC-*N*-oxide fumarate and E-DEC HCl for the high temperature conditions of GLC analysis. The onset of melting for DEC citrate, DEC-*N*-oxide fumarate and E-DEC HCl were 136.4 °C, 127.9 °C and 188.6 °C, respectively (Lee *et al.*, 1997).

A GC method using flame ionization detection was used for the determination of DEC in human plasma. DEC and the internal standard, 1-diethylcarbamy-4-ethyl piperazine HCl (E-DEC), were extracted from human plasma after loading onto a conditioned C₁₈ solid phase extraction cartridge, rinsed with water and eluted with methanol. Samples were evaporated under a gentle stream of nitrogen. When evaporation was complete samples were reconstituted with 50 µl of methanol, 3 µl were injected onto the GC system. Separation was achieved on a A Heliflex[®] AT-35 capillary column (length 30 m, internal diameter 0.32 mm). The GC was operated at the following temperatures: injector, 180 °C; oven, 160 °C; and the detector at 240 °C. Gas flow rates were: hydrogen, 35 ml/min; carrier gas (helium), 1.5 ml/min, make-up gas (helium), 25 ml/min; and air 420 ml/min. The retention times of DEC and internal standard were approximately 5.5 and 7.28 min, respectively. The assay was linear in concentration range of 100 to 2,000 ng/ml for DEC in human plasma (n=6). The analysis of quality control samples for DEC (120, 1000 and 2000 ng/ml) demonstrated precision with CV were 4.5, 1.3, and 1.6%, respectively (n=6). The accuracy was showed with all intra-day (n=6) and inter-day (n=12) within 4.3%. The recovery of DEC and E-DEC were 81.3% to 90.9% and 82.4% to 90.7%, respectively. DEC was found to be stable after 3 freeze-thaw cycles and with storage at -20 °C for 12 weeks (Miller *et al.*, 2001).

A simple and reproducible method for the estimation of DEC by HPLC in DEC-medicated salt was developed. HPLC analysis was conducted with a mobile phase containing acetonitrile/phosphate buffer (20 mM KH₂PO₄, adjusted to pH 3.2 with 10% ortho-phosphoric acid) in the ratio 10:90 and at flow rate of 1.5 ml/min. Analysis was done at UV 210 nm, 0.02 A.U.F. and 40 °C. The CV was 3.1-3.9% (in aqueous; n=5). The quantity of DEC-medicated salt prepared by two methods was analyzed by using HPLC method. A total of 196 DEC-medicated salt samples; 134 from rotating drum method and 62 from spray drying method were analyzed for DEC content. In spray drying method, 29 and 71% of the samples and in rotating drum method, 9

and 12% of samples were found to contain DEC at 0.15-0.25% and >0.25%, respectively (Mathew and Kalyanasundaram, 2001).

Chronopharmacokinetic of DEC was studied. This study was conducted in twelve healthy volunteers by administering a 150 mg single oral dose of DEC at 06.00 or 18.00 h in a balanced crossover design. Plasma DEC concentration was estimated using HPLC with an electrochemical detector. Recovery from liquid extraction of DEC and internal standard (quinidine sulfate) were 90.0-96.2%. CV of inter-day and intra-day were 3.6-6.5%. The mean \pm SD values of pharmacokinetic parameters of DEC for the treatments at 06.00 vs. 18.00 h were as follows: C_{\max} , 500 ± 227 vs. 637 ± 401 ng/ml; $t_{1/2,\lambda_z}$, 14.6 ± 6.7 vs. 11.4 ± 4.9 h and $AUC_{0 \rightarrow \infty}$, $5,840 \pm 1,922$ vs. $7,220 \pm 4,205$ ng.h/ml. None of the parameters was significantly changed ($P \leq 0.05$) (Bolla *et al.*, 2002).

Rifampicin

Rifampicin (Figure 2) 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, 1996).

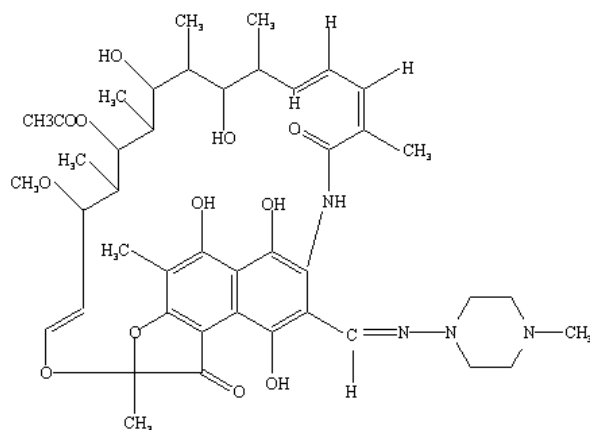


Figure 2 Structure of rifampicin

Chemical and Physical Properties

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

Molecular weight : 822.96

pK_a : 1.7, 7.9

(Windholz *et al.*, 1976)

Solubility :

in water, acetone, alcohol and ether : slightly soluble

in chloroform : freely soluble

(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*; bacteriocidal concentrations range from 3 to 12 ng/ml. The drug is also 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 $\mu\text{g/ml}$. The majority of strains of *M. scrofulaceum*, *M. intracellulare*, and *M. avium* are suppressed by concentrations of 4 $\mu\text{g/ml}$, but certain strains may be resistant to 16 $\mu\text{g/ml}$. *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 were 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 µg/ml, but there is considerable variability. Aminosalicic 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 h 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 does 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 h, reduce its C_{max} from 8.0 to 3.8 µg/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 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 decrease its absorption (Siegler *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 minimum inhibitory concentration (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 V_d (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 concentration is 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 concentration is attained. The pathway of rifampicin metabolism, and metabolic derivatives are illustrated in Figure 3 (Holdiness, 1984).

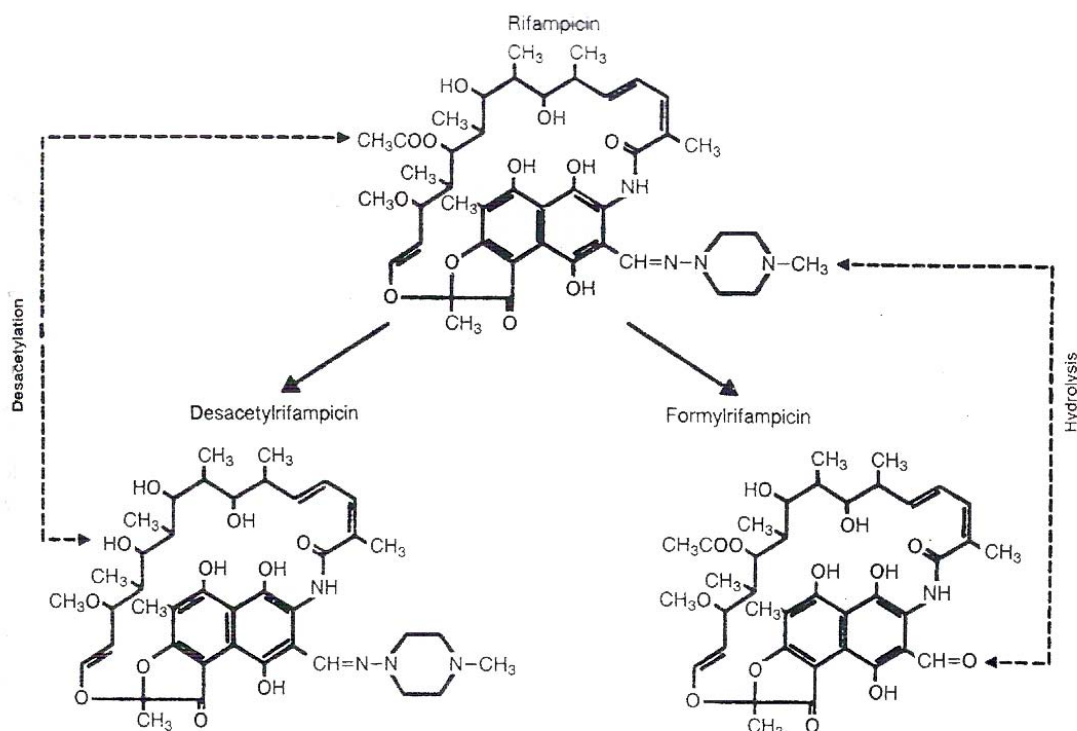


Figure 3 Principal metabolic derivatives of rifampicin in man (Holdiness, 1984).

Figure 3 presents the metabolic routes of rifampicin in man, the major pathway being 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 metabolic pathway, hydrolysis yields a 3-formyl-rifampicin derivative. The deacetylated form 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, para-nitrophenol glucuronyltransferase, β -N-acetylglucosaminidase, and corticosteroid hydroxylase (Holdiness, 1984).

It has also been demonstrated recently that 15 to 20% of deacetylriofampicin 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 h 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 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 dialyzable 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 h 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 $\mu\text{g}\cdot\text{h}/\text{ml}$ (normals) and 105.2 $\mu\text{g}\cdot\text{h}/\text{ml}$ (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 h 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 h in the newborns compared with 2.5% of 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 dose of 10 mg/kg. The C_{max} and $t_{1/2}$ of rifampicin were $8.83 \pm 1.72 \mu\text{g/ml}$ and $4.09 \pm 2.59 \text{ h}$, 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 $t_{1/2}$ ($4.65 \pm 2.61 \text{ h}$) values of desacetyl rifampicin. However, the renal clearance of rifampicin ($7.5 \pm 3.6 \text{ ml/min}$) and desacetyl rifampicin ($14.6 \pm 2.7 \text{ ml/min}$) during a 24-hour period were lower than those observed

in younger individuals (rifampicin, 30 ± 7.6 ml/min; desacetyl-rifampicin, 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 does not appear to be associated with clinically important problems and is not a contraindication to breast feeding (Mcnicol *et al.*, 1995).

4. Adverse effects

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%), 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 (INH) 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 of a number of compound, 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 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 therapy, 600 mg of rifampicin is given twice weekly. Rifampicin is also 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 interactions 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 doses 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 concentration 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 fold, 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 co-administration of rifampicin did not significantly alter the pharmacokinetic parameters of propafenone, *N*-desalkylpropafenone, or propafenone glucuronide after intravenous administration; only the AUC and Ae (amount excretion into the urine) 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.

Celiprolol

Ten healthy volunteers received a 5-day pretreatment with rifampicin (600 mg daily) or placebo. On day 6, a single 200-mg dose of celiprolol was administered orally. Rifampicin reduced the median $AUC_{0 \rightarrow 33}$ of celiprolol 0.44-fold compared with the placebo. The median celiprolol C_{max} was non-significantly (0.66-fold) reduced by rifampicin. The t_{max} and elimination $t_{1/2}$ of celiprolol were not changed by the rifampicin pretreatment. During the rifampicin phase, the median cumulative excretion of celiprolol into urine was reduced to 0.53-fold and celiprolol renal clearance increased 1.19-fold, relative to the placebo phase (Lilga *et al.*, 2004). In vitro studies suggest that celiprolol is a substrate for P-glycoprotein and possibly for some other transporter proteins (Karlsson *et al.*, 1993).

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 ethinyloestradiol.

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 nephritic

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 V_d nor the protein binding of prednisolone was significantly altered.

6.5 Hypoglycaemics

Tolbutamide

Syvalahti *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 co-administration. 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 h. 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 h 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 h. 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).

Gliclazide is extensively metabolized by cytochrome P450 (CYP) 2C9, with less than 20% of the administered dose recovered as the parent form in urine. Nine healthy subjects were treated once daily for 6 days with 600 mg rifampicin or with placebo. On day 7, a single dose 80 mg gliclazide was administered orally. Rifampicin is a potent inducer of CYP3A4 in both the liver and intestine. Furthermore, drug interaction studies show that rifampicin also induces CYP2C enzymes, including CYP2C9. Rifampicin significantly affected the pharmacokinetics of gliclazide. The mean $AUC_{0 \rightarrow \infty}$ of gliclazide was decreased by 70% by rifampicin. The elimination half-life of gliclazide was shortened from 9.5 h to 3.3 h, and C_{\max} was also decreased, by 43%. Cl/F increased about 4-fold after rifampicin treatment compared with placebo treatment (Park *et al.*, 2003).

Niemi *et al.* (2003) showed that administration of rifampicin 600 mg for 5 day could significantly decreases the plasma concentrations of both nateglinide and its M7 metabolite. The mean $AUC_{0 \rightarrow 7}$ of nateglinide declined by 24% ($P = 0.0009$). Rifampicin had no significant effect on the mean C_{\max} of nateglinide. The $AUC_{0 \rightarrow \infty}$ of nateglinide decreased by 22% by treatment ($P = 0.00004$) and shortened its $t_{1/2}$ from 1.6 to 1.3 h ($P = 0.001$). Rifampicin also reduced the $AUC_{0 \rightarrow \infty}$ of M7 by 22% ($P = 0.004$) and shortened its $t_{1/2}$ from 2.1 to 1.6 h ($P = 0.008$).

Rifampicin caused several drug interactions with co-administered drugs. Park *et al.* (2004) studied effect of rifampicin on the pharmacokinetics of rosiglitazone in healthy subjects. Result showed that rosiglitazone was significantly decreased the $AUC_{0 \rightarrow \infty}$; 2947.9

ng.h/ml versus 991.5 ng.h/ml ($P < 0.01$), the $t_{1/2el}$; 3.9 hours versus 1.5 hours ($P < 0.01$), and the C_{max} ; 537.7 ng/ml versus 362.3 ng/ml ($P < 0.01$) after rifampicin phase. In addition, the Cl/F of rosiglitazone increased about 3-fold (2.8 L/h versus 8.5 L/h, $P < 0.001$) after post-treatment also. These results may be caused the interaction between rifampicin and rosiglitazone that probably involves the induction of CYP2C8 and, to a lesser extent, CYP2C9.

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) 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 co-administration 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 cyclosporine 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.

Co-administration 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. Co-administration of rifampicin results in undetectable levels of itraconazole in all subjects except one normal volunteer. The mean AUC_{0-24} was 0.39 vs 3.98 $\mu\text{g/ml}\cdot\text{hr}$ with and without 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.

Stone *et al.* (2004) studies drug-interaction between caspofungin and nelfinavir or rifampin. In study A, healthy subjects received a 14-day course of caspofungin alone (50 mg administered intravenously [IV] once daily) or with nelfinavir (1,250 mg administered orally twice daily) or rifampin (600 mg administered orally once daily). In study B, 14 subjects received a 28-day course of rifampin (600 mg administered orally once daily), with caspofungin (50 mg administered IV once daily) co-administered on the last 14 days, and 12 subjects received a 14-day course of caspofungin alone (50 mg administered IV once daily). When caspofungin and rifampin were initiated on the same day, there were statistically significant ($P < 0.001$) elevations in $AUC_{0\rightarrow 24}$ and C_{24h} of 61 and 170%, respectively, on day 1, but not day 14 in the panel receiving caspofungin with rifampin compared to the panel receiving caspofungin alone.

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 co-administration 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/day. 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/day, and indinavir, 800 mg every 8 h, 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/day, 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 co-administration of amprenavir and rifampicin resulted in significant changes in the pharmacokinetics of amprenavir, including an 82% decrease in the AUC 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 h for 30 days; 7 of the patients also received rifampicin, 600 mg/day, on days 16 to 30 of delavirdine therapy. In the rifampicin group, oral clearance of delavirdine increased nearly 27-fold, and plasma 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.

Nucleotide reverse transcriptase inhibitor

All subjects were given tenofovir DF at 300 mg once a day from day 1 to 10 (period 1). From days 11 to 20 the subjects received tenofovir DF at 300 mg combined with rifampin at 600 mg once daily (period 2). The tenofovir disoproxil fumarate (tenofovir DF) $AUC_{0\rightarrow24}$, C_{max} , and C_{min} were lower in period 2 when tenofovir DF was co-administered with rifampicin. However, the magnitudes of these differences were small, with geometric mean ratios of 0.88 (0.84 to 0.92), 0.84 (0.78 to 0.90), and 0.85 (0.80 to 0.91) for $AUC_{0\rightarrow24}$, C_{max} , and C_{min} , respectively, suggesting pharmacokinetic equivalence when tenofovir DF was dosed with or without rifampicin (Droste *et al.*, 2005).

Enfuvirtide

Boyd *et al.* (2003) have found that rifampicin has no effect on the metabolism of enfuvirtide. The ratios of least squares means (LSM) and 90% confidence intervals for enfuvirtide and enfuvirtide metabolite pharmacokinetic parameters ($AUC_{0\rightarrow12}$, C_{max} , C_{12}) were estimated in the presence and absence of rifampicin. Steady-state rifampicin had no appreciable effect on any of the pharmacokinetic parameters assessed for either enfuvirtide or its metabolite. The ratio of LSM for $AUC_{0\rightarrow12}$, C_{max} , C_{12} for enfuvirtide was 97.5%, 103%, and 84.9%, respectively, and

108%, 112%, and 92.9%, for the enfuvirtide metabolite. These results indicated that enfuvirtide did not inhibit the activities of several cytochrome P450 isoenzymes.

6.11 Psychotropic Agents

Benzodiazepines

Backman *et al.* (1996) studied 10 patients receiving rifampicin, 600 mg/day, or a placebo for 5 days. Midazolam, 15 mg/day 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/day, 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.*, 1997).

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 ± 0.5 to 1.6 ± 0.1 h by rifampicin. The effects of zolpidem are considerably reduced by rifampicin because of enhanced metabolism of zolpidem (Villikka *et al.*, 1997).

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 (60 mg quinine sulphate) was studied in nine healthy young Thai male volunteers. The mean clearance (Cl/F) of quinine co-administered 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 co-administered 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 findings 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

Riditid *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.*, 1997).

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, co-administration 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.

Imatinib

Bolton *et al.* (2004) studied rifampicin-imatinib interaction. Fourteen healthy male or female subjects received imatinib as a single 400 mg oral dose on study day 1 and on study day 8. Rifampicin treatment (600 mg once daily) was initiated on study day 8 and maintained until day 18. During concomitant rifampicin administration, the mean imatinib C_{\max} , $AUC_{0 \rightarrow 24}$ and $AUC_{0 \rightarrow \infty}$ decreased by 54%, 68% and 74%, respectively. The increase in clearance (Cl/F) was 385% after rifampicin pretreatment. The mean C_{\max} and $AUC_{0 \rightarrow 24}$ of the principal metabolite CGP74588 increased by 88.6% and 23.9% after rifampicin pretreatment. However, the $AUC_{0 \rightarrow \infty}$ decreased by 11.7%.

Atorvastatin

Backman *et al.* (2005) indicated that rifampin markedly decreases the plasma concentrations of atorvastatin and its metabolites. Atorvastatin (acid) is biotransformed to atorvastatin lactone, which has been proposed to occur via a coenzyme A-dependent or an acyl glucuronide pathway. Both atorvastatin and its lactone form are metabolized primarily by cytochrome P450 (CYP) 3A4 to hydroxylated metabolites. The lactone forms of atorvastatin and its metabolites can also be hydrolyzed to their acid forms nonenzymatically or by esterases and

paraoxonases. Rifampin 600 mg or placebo was administered in ten healthy volunteers (once daily for 5 days). On day 6, they ingested a single 40-mg dose of atorvastatin. Rifampin decreased the $AUC_{0 \rightarrow \infty}$ and $t_{1/2}$ of unchanged atorvastatin (acid) and its metabolites. The $AUC_{0 \rightarrow \infty}$ of atorvastatin was decreased by 80% ($P < 0.001$) and the C_{max} was decreased by 40% ($P = 0.003$) by rifampin compared with the placebo phase. The AUC values of the active metabolites 2-hydroxyatorvastatin acid and 4-hydroxyatorvastatin acid were 43% and 81% smaller during the rifampin phase than during the control phase ($P < 0.001$). However, the C_{max} of 2-hydroxyatorvastatin acid was increased by 68% by rifampin ($P = 0.005$). Rifampin considerably shortened the half-lives of atorvastatin, 2-hydroxyatorvastatin acid, and 4-hydroxyatorvastatin acid ($P < 0.001$). The $AUC_{0 \rightarrow \infty}$ values of atorvastatin lactone, 2-hydroxyatorvastatin lactone, and 4-hydroxyatorvastatin lactone were reduced by 93%, 61%, and 76%, respectively, and their half-lives were greatly shortened by rifampin ($P < 0.001$). These results strongly suggested that rifampin induced the CYP3A4-mediated first-pass metabolism of parent drug because it was metabolized by CYP3A4.

Nilvadipine

Saima *et al.* (2002) studied the effects of rifampicin on the pharmacokinetics and pharmacodynamics of orally administered nilvadipine to healthy subjects. This study found that the mean peak plasma concentrations for nilvadipine before treatment with rifampicin was approximately 20 times greater than that after treatment ($P < 0.05$). According to, the mean AUC for nilvadipine before rifampicin treatment was approximately 30 times greater than that after treatment ($P < 0.05$). Thus, mean apparent oral clearance (Cl/F) for nilvadipine was increased significantly ($P < 0.05$) by treatment with rifampicin. While the administration of nilvadipine elicited a significant ($P < 0.05$) decrease in the diastolic blood pressure and a significant response to nilvadipine were observed after the treatment with rifampicin. These results showed a possible mechanism for the drug interaction between rifampicin and nilvadipine could be induction by the former of hepatic and intestinal CYP3A4 activity as has been shown for other calcium channel, including verapamil (Fromm *et al.*, 1998) and nefidipine (Holtbecker *et al.*, 1996).

Praziquantel

Riditid *et al.* (2002) found that rifampin greatly decreased plasma concentrations of single and multiple oral doses of praziquantel to levels lower than that of the minimum therapeutic concentration. In the single-dose study, the mean C_{max} and $AUC_{0 \rightarrow 24}$ significantly

decreased by 81% ($P < 0.05$) and 85% ($P < 0.01$), respectively after rifampin pretreatment in 3 subjects (Undetectable plasma level of praziquantel in 7 of 10 subjects). Whereas the multiple-dose study, rifampin significantly decreased the mean C_{\max} and $AUC_{0 \rightarrow 24}$ of praziquantel by 74% ($P < 0.05$) and 80% ($P < 0.01$), respectively (Undetectable plasma level of praziquantel in 5 of 10 subjects).

Ketoconazole

Ketoconazole is *c/s*-1-acetyl-4-(*p*-[[2-(2, 4-dichlorophenyl)-2-imidazol-1-ylmethyl] 1, 3-dioxolan-4-yl] methoxy] phenyl] piperazine (Cordoba-Diaz *et al.*, 2001). The structural formula of ketoconazole is shown in Figure 4. Ketoconazole is the first orally absorbable antifungal azole, was introduced in 1970 (Lyman and Walsh, 1992). It offered a number of significant advantages, including its broad spectrum of antifungal activity, possesses some antibacterial activity (Shuster, 1984; McGrawth and Murphy, 1991) and wide tissue distribution. But it possesses strong inhibitory effect on cyclosporin oxidase and testosterone 6 β -hydroxylase activity in human (Baldwin *et al.*, 1995).

1. Chemical and Physical Properties

Chemical structure : $C_{26}H_{28}Cl_2N_4O_4$

Molecular weight : 531.4

pK_a : 2.94, 6.51

Solubility

In alcohol : 1 in 54 (w/v)

In water : almost insoluble

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

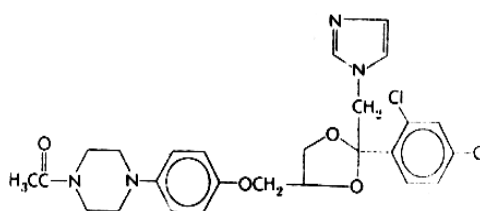


Figure 4 Structural formula of ketoconazole

2. Pharmacokinetics

2.1 Absorption

Ketoconazole is more rapidly absorbed and produces higher concentrations in plasma when administered to infants and children as a suspension than as a powder mixed with applesauce (Ginsburg *et al.*, 1983). Oral absorption of ketoconazole varies among individuals and bioavailability of tablet is 75% (Koch, 1983; Graybill and Drutz, 1980; Chambers, 2001). Since

an acid environment is required for the dissolution of ketoconazole, bioavailability is markedly depressed in patients taking H₂-histamine receptor blocking agents such as cimetidine or proton pump inhibitors thus these drugs should be taken at least 2 h before ketoconazole (Chambers, 2001; Van Der Meer *et al.*, 1980). Ingestion of food has no significant effect on the maximal concentration of the drug achieved in plasma (Daneshmend *et al.*, 1984). After oral dose of 200 and 800 mg, peak plasma concentrations of ketoconazole are approximately 4.8 and 20 µg/ml, respectively.

Ketoconazole is a lipophilic with poor water solubility except at low pH (pH < 3) (Van Der Meer *et al.*, 1980). Peak serum concentrations of ketoconazole occur within 1 to 4 h (Prod Info Nizoral[®] tablets, 1996).

2.2 Distribution

The drug is widely distributed throughout the body in animal and human. However, the volume of distribution was only 0.36 L/kg (Van Tyie, 1984). In blood, 84% of ketoconazole is bound to plasma proteins, largely albumin and 15% to erythrocytes; and 1% is free (Heel *et al.*, 1982; Chambers, 2001). Ketoconazole is highly distributed into saliva and detectable. It penetrates poorly into CSF and in the CSF of patients with fungal meningitis is less than 1% of the total drug concentration in plasma. In one study, plasma protein binding of ketoconazole was altered in patients with chronic renal disease and hepatic cirrhosis, with the percentage of free ketoconazole markedly increased compared to controls (Martinez-Jorda *et al.*, 1990).

2.3 Elimination

Ketoconazole is extensively metabolized in the liver, the major route of elimination being as metabolites in bile. Ketoconazole itself appears to be oxidized by CYP3A. It does not induce its own metabolism, as clotrimazole does. In three human volunteers given ³H-ketoconazole 2.5 mg/kg about, 70% of the administered dose was excreted within 4 days (57% in feces and 13% in urine). Of the fecal radioactivity, 20-65% was due to unchanged drug and 2-4% of urinary radioactivity (Gascoigne, *et al.*, 1981). There may be enterohepatic circulation because the double peaks plasma concentrations were seen at higher doses of ketoconazole (Brass *et al.*, 1982). Renal insufficiency does not affect the plasma concentration or half-life, but the half-life is prolonged in patients with hepatic insufficiency.

The elimination half-life appeared to be dose-dependent, increasing with increasing dose and after repeated dosing (Daneshmend *et al.*, 1983). With an oral dose of

200 mg, the range of mean ketoconazole half-life is 1.51 to 4 h. At higher dose (400 and 800 mg) the mean half-life was 3.7 h (range from 1.3 to 11.6 h) (Maksymink *et al.*, 1982).

3. FDA Labeled Uses

3.1 African histoplasmosis

3.2 Candidiasis-chronic mucocutaneous, Candidiasis-disseminated, candidiasis-esophageal, Disseminated Candida sepsis, Esophageal candidiasis, Mucocutaneous candidiasis, Oral candidiasis in AIDS, Oropharyngeal candidiasis

3.3 Cavitory histoplasmosis

3.4 Chromomycosis systemic infection

3.5 Coccidioidal meningitis, Coccidioidomycosis

3.6 Cutaneous dermatophyte infections

3.7 Dermatitis-seborrheic, Dermat mucosis

3.8 Disseminated histoplasmosis, Histoplasmosis in AIDS

3.9 Paracoccidioidomycosis

3.10 Pityriasis versicolor

3.11 *Seborrheic dermatitis*

3.12 Thrush

3.13 *Tinea cruris, Tinea versicolor*

4. Therapeutic use

4.1 Acne

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

4.2 Arthritis

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

4.3 Athlete androgen administration test

The suppressive effects of ketoconazole on testicular androgen production demonstrated an effective test to distinguish testosterone and other androgen administration in a study involving testosterone pretreated male subjects and untreated healthy male subjects. Study participants received ketoconazole 400 mg at 0 and 2 h after sampling and were enrolled in five separate study groups. The testosterone treated subjects included the subjects receiving ketoconazole on day 3 (n=9), subjects receiving ketoconazole on day 10 (n=9), and subjects with mild primary hypogonadism (n=5) on stable testosterone medication receiving ketoconazole on day 8. The two untreated groups were administered ketoconazole on day 3, the controls (n=9) and athletes that had been previously tested three times with a naturally high testosterone to epitestosterone ratio (T/EpiT) (n=5). Serum testosterone and urinary T/EpiT ratio were evaluated every two hours for an 8 h time period. A significant difference was evident between the testosterone treated groups and the untreated groups ($P < 0.0001$). The serum testosterone concentration remained unchanged and the T/EpiT ratio increased in the testosterone treated study groups after the administration of ketoconazole, whereas the serum testosterone concentration and T/EpiT ratio decreased by 90% and 60%, respectively, after ketoconazole administration in the untreated study groups. The suppressive effects of ketoconazole on endogenous androgen production support a useful and effective test for verifying testosterone and other androgen administration by athletes. In addition, the ketoconazole suppression test may provide an opportunity to distinguish between those athletes with naturally high T/EpiT ratio and those administering testosterone (Oftebro *et al.*, 1994).

4.4 Blastomycosis

Infections with *Blastomyces dermatitidis* are common in the Midwest and Southeastern areas in the US. The most common organs involved are the skin and the lung (Meyer *et al.*, 1993). For the treatment of non-life threatening blastomycosis in immunocompetent patients, the current drug of choice is ketoconazole (Anon, 1992); however, one reference disagrees and the authors feel that itraconazole should be the drug of choice because it is more effective and better tolerated than ketoconazole (Como and Dismukes, 1994). For the treatment of more serious infections or those involving the central nervous system, amphotericin B is considered the drug of choice (Anon, 1992; Mandell *et al.*, 1990). For patients who are immunocompromised such as transplanted patient or patients with AIDS, treatment with

amphotericin B followed by long-term treatment with ketoconazole in doses of 400 to 800 mg daily has been used (Serody *et al.*, 1993; Papaas *et al.*, 1992).

4.5 Candidiasis-cutaneous

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

4.6 Candidiasis-cutaneous

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

4.7 Candidiasis-disseminated

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

4.8 Candidiasis-esophageal

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

4.9 Candidiasis-oral

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

4.10 Candidiasis-urinary

Ketoconazole was used in 11 patients to treat 13 episodes of fungal urinary infections. Eight of the infections were caused by *Candida* species, 1 by mixed *C. tropicalis* and *T. glabrata*, and 3 by *T. glabrata*. Ketoconazole was administered orally at doses of 200 to 800 mg/day for courses ranging from 5 days to more than 2 years. Five episodes of the candida

infections and 1 of the *T. glabrata* infections resolved in association with ketoconazole therapy (Graybill *et al.*, 1983).

4.11 Candidiasis-vaginal

Ketoconazole is not currently FDA approved for the treatment of vulvovaginal candidiasis. The CDC recommends the use of topical antifungal therapy (CDC, 1993). However, in several trials oral ketoconazole has been effective for the treatment of vaginal candidiasis (Talbot and Spencer, 1983; Kovacs *et al.*, 1990; Balbi *et al.*, 1986; Sobel, 1986).

4.12 Chromomycosis-systemic infections

Ketoconazole is indicated for the treatment of systemic chromomycosis infections. The usual oral adult starting dose for chromomycosis infections is 200 mg single dose and may be increase to 400 mg once daily. For children over 2 years old, a single daily dose of ketoconazole 3.3 to 6.6 mg/kg is recommended. Treatment should be continued for a minimum of 6 months (Prod Info Nizoral[®], 1995).

4.13 Coccidioidomycosis

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

4.14 Cushing's disease

Ketoconazole produced a biochemical and hormonal improvement for most patients with Cushing's syndrome secondary to ectopic adrenocorticotropin (ACTH) production by malignant tumors in a retrospective chart review. A total of 15 patients were assessable, including 11 patients with primary lung cancer (9 small cell lung cancer (SCLC), one mixed SCLC/non-SCLC, and one non-SCLC), two metastatic carcinoid tumors (pancreatic and bronchial), one metastatic hepatocellular carcinoma, and one metastatic medullary carcinoma of the thyroid. Patients received ketoconazole orally starting at 400 mg daily in divided doses and titrated according to 24-h urinary free-cortisol (UFC) levels up to 1,200 mg daily for a median duration of 26 days. Concurrent combination chemotherapy was administered to nine patients. Complete response (CR) was defined as normal post treatment UFC levels or normalization of morning plasma cortisol levels if the UFC levels were not available. Partial response (PR) was considered a UFC reduction to less than 50% of baseline, and all other results were defined as no response (NR). Ten of the 12 assessable patients demonstrated a hormonal response, including seven CR

and three PR (median duration 25 days). Clinical and biochemical improvement occurred in most patients, including hypokalemia, metabolic alkalosis, diabetes, and hypertension. Four patients experienced hypoadrenalism (three definite and one probable) and possibly were associated with the level of hormonal control. The cortisol response to stress may be diminished in patients with good hormonal control secondary to ketoconazole therapy, supporting the role of prophylactic replacement corticosteroids in these patients, and the administration of moderate to high-dose corticosteroids for potential stress situations. Progressive malignant disease was responsible for death in most patients (median survival 19 weeks), accompanied by worsened hypercortisolemia despite ketoconazole therapy (Winqvist *et al.*, 1995).

4.15 Cutaneous dermatophyte infections

Oral ketoconazole is indicated for the treatment of cutaneous dermatophyte infections that have not responded to topical therapy or oral griseofulvin or those unable to tolerate griseofulvin. Patients received 100 to 400 mg oral ketoconazole daily for an average of 2 months, although 8 months of duration have been observed (Robertson *et al.*, 1980; Welsh and Rodriguez, 1980).

4.16 Dermatitis-seborrheic

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

4.17 Histoplasmosis

Ketoconazole may be used to treat less serious histoplasmosis infections, but for infections in immunocompromised host or for serious infections, amphotericin B should be used. For patients with AIDS, long-term suppressive therapy is necessary to prevent recurrence. An oral azole (such as ketoconazole, itraconazole, or fluconazole) or amphotericin B in a weekly or biweekly schedule has been used for maintenance therapy (Neubauer and Bodensteiner, 1992). Ketoconazole 400 mg daily for 12 months has been recommended in adults with progressive disseminated histoplasmosis (Hawkin *et al.*, 1981).

4.18 Ovarian hyperandrogenism

Ketoconazole has been recommended to be used only in selected patients with non-tumoral hyperandrogenism because of adverse effects. In a study of 37 women with

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

4.19 Paracoccidioidomycosis

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

5. adverse effects

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

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

discontinue the drug before major surgical procedures and to avoid using high doses in patients with trauma, severe burn, of other stressful conditions.

Mild, asymptomatic elevation of aminotransferase activity in plasma is common occurring in 5% to 10% of patients; these values revert to normal spontaneously. Symptomatic drug induced hepatitis is rare but is potentially fatal. Hepatitis may occur after a few days of treatment, or it may be delayed for many months. The earliest symptoms are anorexia, malaise, nausea, and vomiting, with or without dull abdominal pain. Liver function tests usually mimic the pattern seen with hepatitis A. But a cholestatic or mixed picture can occur. Patients should be alerted to the symptoms and asked to return for liver function tests should this toxicity be suspected. Ketoconazole is teratogenic in animals, causing syndactylus in rats. Its use during pregnancy is not recommended, and because of secretion of the drug into breast milk, its use in nursing mothers also is warning (Chambers, 2001).

6. Contraindication

A. Hypersensitivity to ketoconazole.

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

C. Concurrent use with astemizole [Since, enzyme inhibiting drugs such as ketoconazole may lead to high levels of astemizole if used concurrently. Astemizole overdoses have led to prolonged QT interval and severe ventricular arrhythmias (Hoppu *et al.*, 1991; Snook *et al.*, 1988)] terfenadine [Concomitant use of terfenadine and ketoconazole is contraindicated (Prod Info Nizoral[®], 1998). Co-administration may result in QT prolongation due to inhibition of terfenadine metabolism (Mathews *et al.*, 1991; Eller *et al.*, 1991; Honig *et al.*, 1993)], cisapride [Concomitant administration of cisapride and ketoconazole has resulted in marked increases in cisapride plasma concentrations and prolonged QT interval.

7. Precautions

7.1 Impaired hepatic function

7.2 Impaired adrenal reserve

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

7.4 Patients with achlorhydria

8. Drugs Interaction

8.1 Oral Anticoagulants

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

8.2 Benzodiazepines

Chlordiazepoxide is extensively oxidized in the liver with little urinary excretion of the parent drug. Ketoconazole impaired chlordiazepoxide clearance from plasma. After a single dose of ketoconazole there was a 20% decrease in clearance and 26% decrease in volume of distribution without evidence of inhibition of drug metabolism. These changes apparently were not related to ketoconazole dose. After repetitive dosing with ketoconazole, chlordiazepoxide clearance decreased by 38% and was associated with reduced concentrations of its first oxidative metabolite, N-desmethylchlordiazepoxide. It was concluded that ketoconazole inhibits at least one subset of the hepatic mixed-function oxidase system, but not generally (Brown *et al.*, 1985).

Concomitant use of ketoconazole and alprazolam may result in increased serum concentrations of alprazolam and associated alprazolam toxicity (excessive sedation, fatigue, ataxia, slurred, slowed reactions and other psychomotor impairment). *In vitro* studies have shown ketoconazole to be a potent inhibitor of cytochrome P450 3A (CYP3A) enzymes, an enzyme subfamily thought to be important in alprazolam metabolism (von Moltke *et al.*, 1994; Greenblatt *et al.*, 1993; Greenblatt *et al.*, 1998). Because the initial step in alprazolam metabolism is hydroxylation catalyzed by CYP3A, ketoconazole may have a profound effect on the clearance of alprazolam. Concomitant administration of these two agents is contraindicated (Prod Info Xanax[®], 1997).

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

The role of P-glycoprotein (P-gp) on the distribution of the benzodiazepine, triazolam, and the azole antifungal agent ketoconazole, and on the triazolam-ketoconazole interaction, was studied using *mdr1a*(-) or *mdr1a/b*(-/-) mice (P-gp-deficient mice) and matched controls. Compared with animals receiving triazolam alone, co-administration of triazolam with ketoconazole caused an elevation of serum, brain, and liver concentrations of triazolam. This was true in both FVB controls and *mdr1a*(-) animals. Brain/serum triazolam ratios, although slightly lower in animals co-treated with ketoconazole, were not significantly different between triazolam alone and triazolam plus ketoconazole groups, either in FVB controls or in *mdr1a*(-) mice. Likewise, liver/serum ratios were not significantly changed by co-treatment of ketoconazole (von Moltke *et al.*, 2004).

Substantial increases in oral midazolam peak plasma concentration (310%), AUC (1490%), and half-life (210%) have been demonstrated to occur with concurrent oral ketoconazole compared to placebo in healthy volunteers (Olkola, *et al.*, 1994). Psychomotor tests and subjective reporting of drowsiness with the combination indicated significant increases in sedative effects. Oral midazolam is not recommended for patients receiving ketoconazole. Ketoconazole is a known inhibitor of the cytochrome P450 3A4 (CYP3A4) enzyme system, and midazolam metabolism is mediated through CYP3A4. Co-administration of these two agents may result in prolonged sedation due to reduced midazolam plasma clearance (Prod Info Versed[®], 1997). Inhibited CYP3A activity caused by ketoconazole appears to be greater in the intestine than in the liver (Tsunoda *et al.*, 1999).\

8.3 Calcium channel blocking agents

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

8.4 Amphotericin B

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

8.5 Tirilazad

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

U-87999), which suggests that all of the compounds are substrates for CYP3A (Fleishaker *et al.*, 1996).

8.6 Quinine

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

8.7 Quinidine

Ketoconazole is well known to inhibit potently CYP3A4 activity. However, ketoconazole inhibits competitively the metabolic activity of CYP3A12, a major isoenzyme of CYP3A subfamily in canine hepatic microsomes (Kuroha *et al.*, 2002). Quinidine (QN) was used as a model drug of CYP3A12 substrate. The result shown that higher concentration of QN was observed after than before ketoconazole treatment. The pharmacokinetic parameters of QN estimated by two-compartmental method, when co-administration of ketoconazole, statistically significant was observed in an exponential at distribution phase (α), at elimination phase (β), $t_{1/2\beta}$, V_1 , and k_a of QN phase. The pharmacokinetic parameters of QN estimated by non-compartmental method, when co-administration of ketoconazole, Cl_{tot} , Cl_{oral} , C_{max} , $MRT_{i.v.}$, $MRT_{p.o.}$, MAT were statistically significant of QN phase (Kuroha *et al.*, 2004).

8.8 Reboxetine

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

ratio for R, R(-)-reboxetine to S, S (+)-reboxetine was reduced by ketoconazole administration (12.76 after ketoconazole versus 2.39; $P < 0.003$).

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

8.9 Amprenavir

Twelve individuals received single dose of amprenavir 1200 mg and ketoconazole 400 mg. Maximum concentration (C_{max}) of amprenavir was decreased an average of 16%, but the area under the concentration-time curve (AUC) increased 31%. Amprenavir increased the ketoconazole C_{max} and AUC by 19% and 44%, respectively (Prod Info Agenerase[®], 2000). The significance of this interaction is unknown, but unlikely to be clinically important (Polk *et al.*, 1999).

8.10 Tolbutamide

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

8.11 Antihistamine

Enzyme inhibiting drug such as ketoconazole may lead to high level of astemizole if used concurrently. Astemizole overdoses have led to prolonged QT interval and severe ventricular arrhythmias (Hoppu, *et al.*, 1991; Snook, *et al.*, 1988). Due to the potential for an interaction that could lead to increased astemizole use with ketoconazole is contraindicated (Anon, 1993; Prod Info Hismanal[®], 1998).

8.12 Antacids

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

Carlson *et al.*, 1983).

8.13 Ritonavir and Saquinavir

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

8.14 Oestrogen

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

8.15 Cocaine and opioid

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

dosing studies in primates and humans, chronic ketoconazole treatment does not appear to reduce cocaine or opioid use in humans maintained on methadone (Kosten *et al.*, 2002).

The co-administration with ketoconazole could increase plasma concentration of Levo-acetyl- α -methadol (LAAM), a synthetic opioid, used in replacement maintenance therapy for opioid dependence. The long duration of action of LAAM has been attributed primarily to its sequential *N*-demethylation to two primary metabolites, norLAAM and dinor LAAM, which have more potent α -agonist activity. The *N*-demethylation of both LAAM and norLAAM is performed primarily by cytochrome P450 (CYP) 3A4, with contributions from CYP2B6, CYP3A5, and members of the CYP2C family (8, 9, 18, and 19 all possess some activity). CYP3A4 substrates are often subject to *in vivo* drug interactions. The most dramatic effects of ketoconazole were the increase in the AUC (5.29-fold) and C_{\max} (3.22-fold) of LAAM, along with the extension of the t_{\max} of norLAAM (2.43-fold) and dinorLAAM (11.6-fold). The increase in LAAM concentration occurred without any significant effect on its t_{\max} or $t_{1/2}$. For norLAAM and dinorLAAM, the extension of the t_{\max} was accompanied by decreased C_{\max} , increased AUC, and slightly increased $t_{1/2}$. The urinary excretion of LAAM, norLAAM, and dinorLAAM paralleled the plasma concentration when LAAM was given after placebo or ketoconazole (Moody *et al.*, 2004).

8.16 Cyclosporine

Five male renal allograft patients of mean age 22 ± 2.3 years displayed a mean AUC of cyclosporine before starting ketoconazole to be 1581.8 ± 484 ng.ml/h. Following addition of 50 mg of ketoconazole, the mean AUC of cyclosporine increased to 4946 ± 1006 ng.ml/h ($P < 0.01$) (Abraham *et al.*, 2003).

8.17 Antiarrhythmic drug

Azimilide dihydrochloride is a class III antiarrhythmic drug. It was metabolized by CYP 3A4/5 and CYP 1A1. Ketoconazole (200 mg) or placebo was administered orally every 24 h for 29 days. On day 8, a single oral dose of 125 mg azimilide dihydrochloride was administered at the same time as ketoconazole/placebo. Results indicated that inter-subject variability for each parameter was very similar between two groups and that pretreatment with ketoconazole decreased azimilide oral clearance (14%), and increases azimilide C_{\max} (120%), AUC (16%), and $t_{1/2,z}$ (13%). No change in t_{\max} , V_z/F or plasma protein binding was observed (Mouelhi *et al.*, 2004).

8.18 Immunosuppressant

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

8.19 Retinoic acid

The aforementioned CYP inhibitors (diethyl dithiocarbamate [DEDTC], ketoconazole [KC] and grapefruit juice [GJ]) could change the pharmacokinetics of All-trans-retinoic acid (ATRA). DEDTC was a powerful inhibitor of ATRA metabolism, with an apparent IC_{50} value of 0.12 μ M, whereas the apparent IC_{50} value for ketoconazole (KC), a well-established CYP inhibitor, was 13.5 μ M. On the other hand, grapefruit juice (GJ) has been reported to increase the bioavailability of certain drugs by inhibiting the intestinal CYP enzyme. For infusion of ATRA, the co-administration of i.v. or oral DEDTC did not change the profile of the plasma AUC of ATRA. However, co-administration of oral KC yielded higher plasma ATRA levels than those obtained in the control group and, consequently, higher C_{max} and $AUC_{0\rightarrow 600}$ values were obtained for the rats in this group. For oral administration of ATRA, after the oral administration of 6.4 and 32 mg/kg of DEDTC, no changes in ATRA pharmacokinetics were observed since the plasma level profile and parameter values of ATRA were similar to those obtained in the control group. However, after the oral administration of 320 mg/kg of DEDTC, a pronounced decrease in plasma ATRA was recorded, and the mean C_{max} and $AUC_{0\rightarrow \infty}$ values were approximately 20% and 24% of the corresponding mean values obtained in the control group. The oral administration of KC gave rise to an increase in the plasma levels, $AUC_{0\rightarrow \infty}$, and $t_{1/2}$ values of ATRA, whereas the oral administration of GJ did not affect the pharmacokinetics of orally administered ATRA (Saadeddin *et al.*, 2004).

8.20 Antidiabetic drug

Ketoconazole increased the plasma concentrations of rosiglitazone. After administration of ketoconazole, the mean $AUC_{0\rightarrow \infty}$ of rosiglitazone was increased by 47%. The elimination half-life of rosiglitazone was lengthened from 3.55 h to 5.50 h and C_{max} was also increased by 17%. The Cl/F decreased about 30% after ketoconazole treatment compared with placebo treatment. Ketoconazole is well known to inhibit potently CYP3A4 activity but

rosiglitazone is metabolized through *N*-demethylation and *p*-hydroxylation, mainly by CYP2C8 and to a lesser extent CYP2C9. Therefore, this study revealed that ketoconazole affected the disposition of rosiglitazone in humans, probably by the inhibition of CYP2C8 and CYP2C9 (Park *et al.*, 2004).

8.21 Anticancer agent

The anticancer drug docetaxel is extensively metabolized by CYP3A. Ketoconazole, CYP3A inhibitor, was used the drug-interaction model. Ketoconazole co-administration resulted in a 49% decrease in clearance of docetaxel. The mean (\pm SD) clearance values were 35 ± 11.8 L/h for docetaxel alone and 18.2 L/h in the presence of ketoconazole, respectively (Engels *et al.*, 2004).

9. Factors Affecting the Pharmacokinetics of Ketoconazole

9.1 Influence of Food Intake

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

9.2 Renal Insufficiency

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

9.3 Hepatic Insufficiency

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

Cytochrome P-450 System

1. Introduction

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

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

CYP represents a superfamily of enzymes. They are found in animals, plants, yeast and bacteria. In mammals, some CYP are involved in pathways of steroid biosynthesis and do not metabolize foreign compounds. However, the vast majority of these enzymes, the foreign compound metabolizing CYP, appear to oxidize chemicals that are not normal constituents of the body. CYP are named with the root CYP followed by an Arabic number and upper case letter designating the family and subfamily, respectively. Individual CYP forms are denoted by Arabic number that follows the subfamily letter (i.e., CYP3A4 is family 3, subfamily A, and gene number 4) (Gonzalez and Idle, 1994). The cytochrome P450 proteins are embedded in the lipid bilayer of the smooth endoplasmic reticulum. An important associated protein, NADPH-cytochrome P450 reductase, is also attached to this lipid bilayer in a stoichiometry of about ten P450 molecules to one reductase. 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 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).

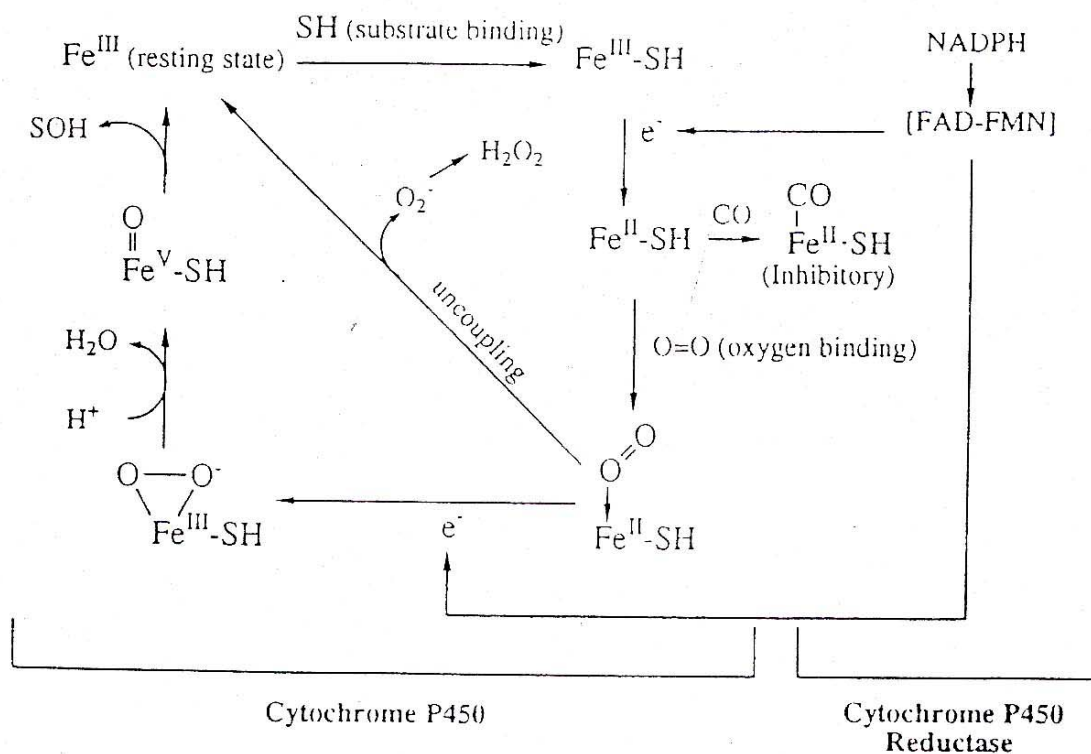


Figure 5 Cytochrome P450 cycle in drug oxidations

2. Human Hepatic Cytochrome P450s (P450s)

The CYP comprise a superfamily of haemoproteins which contain a single iron protoporphyrin IX prosthetic group (Figure 6). This superfamily is subdivided into families and subfamilies that are defined solely on the basis of amino acid sequence homology. In human, 12 CYPs (CYP1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, and 3A5) are known to be important for metabolism of xenobiotic metabolism (Gonzalez and Tukey, 2006). The mammalian CYP families can be functionally subdivided in to 2 major classes, those that involve the biosynthesis of steroids and bile acids and those that primarily metabolize xenobiotics. Three main CYP gene families, CYP1, CYP2 and CYP3 are responsible for most hepatic drug metabolism. Although the CYP1 and CYP3 gene families are relatively simple (i.e. CYP1A, CYP1B and CYP3A), the CYP2 gene family is comprised of many subfamilies (e.g. CYP2A, CYP2B, CYP2C, CYP2D, CYP2E, etc.). These isoforms have the same oxidizing center (the haem iron), but differ by their protein structures (Lin and Lu, 1998).

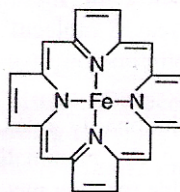


Figure 6 Structure of ferric protoporphyrin IX

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

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

3. Mechanisms of Inhibition of CYP

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

- (i) binding of the substrate to the ferric form of the enzyme
- (ii) reduction of the haem group from the ferric to the ferrous state by an electron provided by NADPH via CYP reductase
- (iii) binding of molecular oxygen
- (iv) transfer of a second electron from CYP reductase and/or cytochrome b5

- (v) cleavage of the O-O bond
- (vi) substrate oxygenation
- (vii) product release

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

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

a. Reversible Inhibition

Many of the potent reversible CYP inhibitors are nitrogen-containing drug, including imidazoles, pyridines and quinolines. These compounds can not only bind to the prosthetic haem iron, but also to the lipophilic region of the protein. Inhibitors that simultaneously bind to both regions are inherently more potent inhibitors. The potency of an inhibitor is determined both by its lipophilicity and by the strength of the bond between its nitrogen lone electron pair and the prosthetic haem iron. For example, both ketoconazole and cimetidine are imidazole-containing compounds that interact with ferric CYP at its sixth axial ligand position to elicit a type II optical difference spectrum. The coordination of a strong ligand to the pentacoordinated iron, or the displacement of a weak ligand from the hexacoordinated haem by strong ligand, gives rise to a “type II” binding spectrum. However, cimetidine is a relatively weak reversible inhibitor of CYP, an apparent result of an intrinsic low binding affinity to microsomal CYP. This latter property is most probably because of the low lipophilicity of cimetidine ($\log P=0.4$). On the other hand, ketoconazole, a potent CYP 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 CYP inhibitor, again due mainly to its low lipophilicity.

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

8-substituent of primaquine is believed to be involved in the haem iron of the ferric CYP (Lin and Lu, 1998).

b. Quasi-Irreversible Inhibition via Metabolic Intermediate Complexation

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

c. Irreversible Inhibition of CYP

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

4. Mechanism of Induction of CYP

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

pharmacokinetic and clinical consequences of CYP induction are often overlooked in clinical studies.

Although the phenomenon of CYP induction has been known for more than 4 decades, only in recent years they had begun to uncover the mechanisms involved in induction. From a biological point of view, induction is an adaptive response that protects the cells from toxic xenobiotics by increasing the detoxification activity. While in most cases CYP induction is the consequence of an increase in gene transcription, some nontranscriptional mechanisms also are known to be involved.

For many years, scientists have been trying to solve the mystery of how the cells recognize the inducing agents and how the signal is transferred to the transcriptional machinery. With the exception of the CYP1A1 isoform, the molecular mechanisms involved in CYP induction are still not fully understood. In the case of CYP1A1, inducing agents bind to cytosolic polycyclic aromatic hydrocarbon receptors (AHR) and are translocated in to the nucleus. The transcriptional process includes a sequence of events: ligand-dependent heterodimerisation between the Ah receptor and an Ah receptor nuclear translocator (AHRT). This heterodimer bind to the xenobiotic response elements (XRE) of CYP genes activating transcription. In other case, such as CYP3A4, inducing agents bind to pregnane X receptor (PXR), PXR forms a heterodimer with the retinoid X receptor- α (RXR α) and transactivates ER6 elements upstream of the CYP3A4 and CYP3A7 genes.

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

5. Clinical Implications

5.1 Inhibition of CYP

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

For example, co-administration of terfenadine, an antihistamine agent, and ketoconazole led to fatal ventricular arrhythmias in some patients. It is metabolized extensively by CYP3A4 in human to form 2 metabolites by N-dealkylation and hydroxylation. After oral administration of a 60 mg dose, terfenadine is usually undetectable in plasma because of extensive first pass metabolism. Concurrent administration of drugs that inhibit terfenadine metabolism can result in an excessive increase in plasma concentration of terfenadine. Clinical data showed that itraconazole and erythromycin also impair the metabolism of terfenadine. Because CYP3A4 represents a major CYP isoform in human liver, and because CYP3A4 has a broad spectrum of substrate specificity, it is likely that many other drugs are capable of inhibiting terfenadine metabolism. Because of its undesirable properties, terfenadine was recently withdrawn from sale or had its use restricted in several countries.

Inhibition can also reduce clinical efficacy, if the drug is a prodrug requiring metabolic activation to achieve its effects and activation is blocked. Reversible enzyme inhibition is transient; the normal function of CYP enzymes continues after the inhibitor has been eliminated from the body. In contrast, the loss of enzyme activity caused by irreversible inactivation persists even after elimination of inhibitor, and de novo biosynthesis of new enzymes is the only mean by which activity can be restored. Clearly, clinical and pharmacokinetic consequences of irreversible drug inhibition are quite complicated, depending on the duration and frequency of administration. The long-term effects of irreversible inhibition on CYP are yet unknown and further studies need to address this question.

Metabolic drug interaction is usually regarded as potentially dangerous, or at least undesirable. However, there are times when these interactions may be exploited. For example, because these 2 drugs are substrates for the same human CYP3A4, the antifungal agent

ketoconazole is used with cyclosporin, an immunosuppressive agent, to prolong the elimination of the cyclosporin. The idea is to use the relatively inexpensive ketoconazole to specifically inhibit the metabolism of the very expensive cyclosporin, thereby minimizing the cost of long-term immunosuppressive therapy. Keogh *et al.* (1995) have reported that ketoconazole reduced by 80% the dose of cyclosporin needed to maintain target concentrations in patients after cardiac transplantation, with a cost saving per patient of approximate \$US 5200 in first year.

5.2 Induction of CYP

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

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

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 inducer, increasing the drug dosage can circumvent the reduction in drug concentration. However, if dosages are increased, there is a danger of excessive accumulation of drug when the inducer is withdrawn enzyme activity returns to normal (Lin and Lu, 1998).

Flavin-containing monooxygenase (FMO)

1. Introduction

Flavin-containing monooxygenase (FMO) comprise a family of FAD-, NADPH- and molecular oxygen-dependent microsomal enzyme. Although FMO has been previously viewed as minor contributors to drug metabolism, the advantages associated with using FMO to diversify the metabolism of a drug are now being recognized. Because FMO typically oxygenate a wide variety of nucleophilic compounds to polar, benign metabolites, and because drugs do not induce expression of FMO or inhibit their activity, potential drug-drug interactions are minimized. Interindividual variation for this class of enzyme is largely dependent on genetic variation. Examples of FMO allelic variation and splicing variants suggest that these genetic mutations could contribute to the interindividual and interethnic variability of FMO-mediated metabolism (Cashman, 2004).

In general, cytochrome P450 (CYP) is the major contributor to oxidative xenobiotic metabolism. However, FMO activity may be of significance in a number of cases and should not be overlooked. FMO and CYP have overlapping substrate specificities, but often yield distinct metabolites with potentially significant toxicological/pharmacological consequences (Kruger and Williams, 2005).

2. Catalytic cycle of FMO

The mechanism of catalysis by FMO is depicted in Figure 7. After the FAD moiety is reduced by NADPH, the oxidized cofactor, NADP^+ , remains bound to the enzyme, which then binds oxygen to produce peroxide (i.e., the 4a-hydroperoxyflavin of FAD). The peroxide is relatively stable, probably because the active site of FMO comprises nonnucleophilic, lipophilic amino acid residues. During the oxygenation of xenobiotics, the 4a-hydroperoxyflavin is converted to 4a-hydroxyflavin with transfer of the flavin peroxide oxygen to the substrate (depicted as $\text{X} \rightarrow \text{XO}$ in Figure 7). The final step in the catalytic cycle involves dehydration of 4a-hydroxyflavin (which restores FAD to its resting, oxidized state) and release of NADP^+ . This final step is important because it is rate limiting, and it occurs after substrate oxygenation. Consequently, this step determines the upper limit of the rate of substrate oxidation. Therefore all good substrates for FMO are converted to products at the same maximum rate (i.e., V_{\max} is determined by the final step in the catalytic cycle). Binding of NADP^+ to FMO during catalysis is important because it prevents the reduction of oxygen to H_2O_2 . In the absence of bound NADP^+ ,

FMO would function as an NADPH-oxidase that would consume NADPH and cause oxidative stress through excessive production of H_2O_2 (Parkinson, 1996).

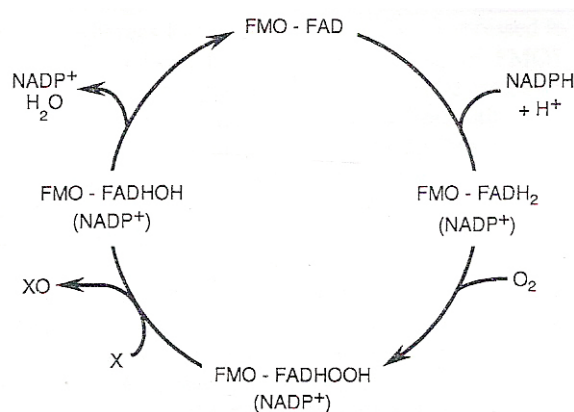


Figure 7 Catalytic cycle of FMO

3. Distribution of FMO forms in animals and humans

Humans and other mammals express five different flavin-containing monooxygenases (FMO1, FMO2, FMO3, FMO4, and FMO5) in a species- and tissue-specific manner. For example, the major FMO expressed in human and mouse liver microsomes is FMO3, whereas FMO1 is the major FMO expressed in rat, rabbit, and pig liver. In humans, high levels of FMO1 are expressed in the kidney, and low levels of FMO2 expressed in the lung. However, lung microsomes from other species, particularly rabbit and mouse, contain high levels of FMO2 (Parkinson, 1996). Based on immunoreactivity investigations, FMO3 is expressed at levels approaching 60% of the expression levels of the major CYP present in adult human liver (CYP3A4) (Cashman, 2004).

4. Role of FMO in drug metabolism

4.1 Role of FMO in metabolism of N-containing drugs

A number of N-containing xenobiotic is the FMO-mediated substrate. Tertiary amines, produced by FMO-mediated N-oxygenation, are highly lipophilic, easily excreted, and typically exhibit markedly less pharmacological and/or toxicological properties than the parent amine or CYP-mediated metabolites. For this reason, FMO-mediated N-oxygenation of tertiary amines usually represents detoxication. For example, the neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyriding (MPTP) is readily N-oxygenated by FMO to the N-oxide, a non-toxic

easily excreted metabolite. If not N-oxygenated by FMO, MPTP can undergo CYP and monoamine oxidase-catalyzed metabolism to the potent neurotoxins MPDP⁺ and MPP⁺ resulting in parkinsonism. The activity of FMO in the N-oxygenation of MPTP in brain is probably of critical importance and may explain species differences in toxicity. The contribution of FMO N-oxygenation may be underestimated as the N-oxide can be reduced back to the parent amine by CYP or other reductases. Such is certainly the case for tamoxifen, nicotine, and many other drugs with tertiary amines subject to N-oxygenation. By this mechanism, FMO N-oxygenation provides a reservoir of available parent drug, prolonging its action. It should be emphasized again that aliphatic tertiary amine oxidation by CYP and FMO typically yields distinct product; CYP oxidation results in N-dealkylation, whereas FMO produces exclusively the N-oxide (Kruger and Williams, 2005).

The contribution of FMO to the metabolism of secondary amines, such as methamphetamine, may also be underestimated. In this case, it is not reduction back to the parent amine, such as with tertiary amines, but rather the production of metabolites that may be attributed to CYP action. Secondary amines are oxygenated by FMO initially to the N-hydroxy amine followed by a second oxygenation to nitrones. The nitrones are typically rapidly hydrolyzed. The hydroxylated primary amine is then reduced enzymatically to the primary amine, producing the same product as CYP-mediated N-dealkylation. Unlike the N-oxygenation of tertiary amines, FMO-mediated metabolism of secondary amines does not always result in detoxication. For example, 3,3'-iminodidipropionitrile is a secondary amine that is bioactivated by FMO to the neurotoxic N-hydroxy-3,3'-iminodidipropionitrile. Another example is N-deacetyl ketoconazole which is N-hydroxylated by a number of FMO. FMO-mediated metabolism of N-deacetyl ketoconazole appears to be involved in hepatotoxicity following administration as a antifungal agent (Kruger and Williams, 2005).

Hydrazine can also be excellent substrate for FMO. The initial product observed is the hydrazone, which is subsequently hydrolyzed to form an aldehyde and a dealkylated hydrazine. Both CYP and FMO are capable of carrying out this reaction; however, it appears that CYP oxygenates 1,2-disubstituted hydrazines, whereas FMO exclusively oxygenates 1,1-disubstituted hydrazines. In addition to industrial uses, hydrazines have been used as pharmaceutical agents for a number of years. The use of hydrazines in therapeutics has been limited by toxicity in liver and other organs. The contribution of FMO-mediated oxygenation of

1,1-disubstituted hydrazines to the risk of developing hydrazine toxicities is not known (Kruger and Williams, 2005).

4.2 Role of FMO in metabolism of S-containing drugs

FMO readily catalyzes the S-oxygenation of numerous drugs and other compounds. These include sulfides, thiols and disulfides, thiocarbamides and thioamides, and mercapto-purines, pyrimidines, and imidazoles. Thiols are oxygenated to disulfides through an intermediate sulfenic acid. Disulfides can be further oxygenated to S-oxide. The endogenous substrate cysteamine is oxygenated to the disulfide cystamine (Kruger and Williams, 2005).

Sulfides are typically substrates for FMO and readily converted to the sulfoxide. The S-oxygenation of thioureas, thioether-containing insecticides such as phorate and disulfoton. FMO readily S-oxygenates simple sulfides such as methyl-, ethyl-, 4-chlorophenyl methyl, and diphenyl sulfides. Sulfide-containing drugs, for which FMO is active in S-oxygenation to sulfoxides, include cimetidine, ranitidine, thioridazine, clindamycin, albendazole, and fenbendazole. FMO can catalyze the further oxygenation of the sulfoxide to the sulfone, but often this reaction is more efficiently catalyzed by CYP. In the case of chiral sulfides, the sulfoxylation is often stereoselective. Interestingly, this stereoselectivity is often distinct from that of CYP sulfoxylation of the same achiral substrate (Kruger and Williams, 2005).

Related to the thioureas, or thiocarbamides, are the thioamides such as thioacetamide and thiobenzamide. Thioacetamide and thiobenzamide are potent liver toxicants, and the hepatotoxicity appears primarily due to FMO-mediated S-oxygenation to sulfines and sulfenes (Kruger and Williams, 2005).

5. Conclusion

The FMO is a family of enzymes that like the CYP superfamily evolved during to metabolize many xenobiotics. There are numerous similarities between these monooxygenase systems. Both utilize reducing equivalents from NADPH to reduce the second atom of molecular oxygen to water and incorporate the other atom into the substrate. FMO and CYP located in the endoplasmic reticulum and are present in highest amounts in liver. CYP and FMO are active toward many of the same xenobiotics. However, there are numerous differences. In contrast to CYP, FMO is heat labile and can be inactivated in the absence of NADPH by warming microsomes to 50 °C for 1 minute. Although FMO and CYP share many of the same substrates, the products are usually distinct, e.g., N-oxides from tertiary amines rather than

the N-dealkylation seen with CYP. The use of chemical inhibitors to ascertain the relative contribution of FMO and CYP to microsomal reactions is often complicated by a lack of specificity. For example, cimetidine and SKF 525A, which are well recognized CYP inhibitors, are both substrates for FMO. The metabolic pathways of two drugs (itopride and cisapride) were demonstrated by Mushiroda *et al.* (2000). The result showed that itopride is metabolized by FMO while cisapride is metabolized by CYP3A4.

P-Glycoproteins

1. Introduction

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

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

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

2. MDR gene family

P-gps are encoded by members of a small gene family referred to as the multidrug resistance (MDR) genes. Because of alternative naming schemes that evolved from the

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

3. Structure of P-glycoprotein

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

P-gp, the first known ATP-binding cassette (ABC) transporter, is composed of two homologous and symmetrical cassettes; each contains six transmembrane domains that are separated by an intracellular flexible linker polypeptide loop with an ATP-binding motif (Figure 8). Hundreds of these traffic ATPases have been identified in bacteria, plants, fungi, and animal cells and are important in the movement of a large number of nutrients and waste products. ABC transporters transport virtually any class of substrate, including ions, sugars, amino acids, peptides, and polysaccharides. These membrane transporters typically have four domains: two have up to six membrane-spanning regions and two, located at the cytoplasmic surface, bind ATP and couple its hydrolysis to substrate transport. Most notably in prokaryotes, these individual domains are encoded by separate genes; however, in mammals, they are often encoded by a large single gene such as MDR1. Examples of ABC transporters include the *Escherichia coli* MalEFGK gene, which imports maltose, the *Saccharomyces cerevisiae* STE6, which exports the peptide α -mating factor, and the *Plasmodium falciparum* transporter *pfrndr*, which transports chloroquine and mediates drug resistance.

Sequence analysis revealed that P-gp is made up of 1,280 amino acids with roughly bilateral symmetry; the amino and carboxy halves of the protein each have six transmembrane domains and an ATP-binding region. This structural model for P-gp has been investigated using antibody mapping, site-directed mutagenesis, and biochemical analysis.

Mapping epitope domains with MRK-16, an antihuman monoclonal, demonstrated that the first and fourth predicted loops are extracellular. Similarly, antipeptide antibodies to Glu³⁹⁸-Lys⁴⁰⁸ and Leu¹²⁰⁶-Thr¹²²⁶ recognize their epitopes in permeabilized, but not intact, cells, confirming their predicted intracellular location. Mapping of the topology of cysteine residues into putative intracellular or extracellular loops provided further support for the 12-transmembrane domain model. High-resolution electron microscopy was used to present a model for P-gp that is consistent with the available immunologic and biochemical analysis. At 2.5 nm resolution, P-gp appears to function as a monomer and have a 5 nm central pore, which is closed on the cytoplasmic surface of the plasma membrane forming an aqueous compartment. Two 3 nm intracellular lobes were observed and are consistent with the predicted 200-amino acid nucleotide binding domains. These data agree with the hypothesis that substrate binding and cross-linking agents interact at the cytoplasmic face of the membrane. Biochemical analysis using nickel-chelate chromatography has also suggested that P-gp functions as a monomer. The previous study was examined the secondary and tertiary structure of P-gp using attenuated total reflection Fourier transform infrared spectroscopy. The secondary structure of P-gp was found to contain 32% α -helix, 26% β -sheet, 29% turns, and 13% random coil; no significant alteration in these parameters occurred upon binding of verapamil, ATP, or a nonhydrolyzable ATP analogue (Silverman, 2000).

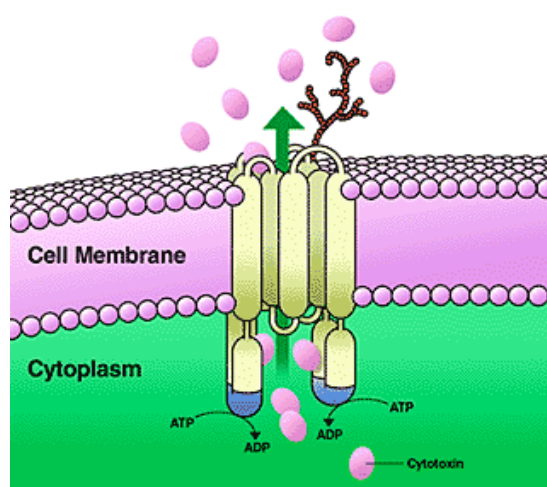


Figure 8 Structure of P-glycoprotein

4. Function of P-glycoprotein

Unlike typical ABC transporters, which have a narrow, usually single substrate range, a defining characteristic of P-gp is its ability to transport literally hundreds of compounds. Increased expression of P-gp is associated with the multidrug-resistant phenotype in which cells become cross resistant to structurally and mechanistically distinct cytotoxic drugs. Demonstration that this protein is responsible for this phenotype comes most clearly from gene transfer experiments. Transfection of high-molecular weight DNA isolated from drug-resistant cells confers a multidrug-resistant phenotype to previously drug-sensitive cells. Similarly, transfection of either the murine *mdr1* or human MDR1 cDNAs into drug-sensitive cells also results in a 200-fold increase in resistance to daunomycin and cross resistance to adriamycin, colchicine, vincristine, and vinblastine. The level of drug resistance in MDR1-transfected cells correlates with the expression of P-gp. Thus transfer of the cDNA-encoding P-gp is in itself sufficient to confer a drug-resistant phenotype upon drug-resistant cells (Silverman, 2000).

5. Role of p-glycoprotein in drug absorption and disposition

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

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

normally express P-gp. The serum terminal half-life of intravenously administered vinblastine was longer in the knockout mice than in wild-type animals, 3.6 versus 2.1 hours, respectively, and the fecal elimination was reduced from 20% to 25% to 9%. Vinblastine also accumulated in the brain, heart, and liver of the *mdr1a*-deficient animals. Similarly, reduced fecal and intestinal elimination and increased tissue accumulation of digoxin was observed in these animals. Thus, P-gp contributes substantially to the elimination of substrate drugs through both hepatic and intestinal secretion.

The *mdr1a* knockout mice have also been used to demonstrate a clear role of P-gp in drug absorption. Increased bioavailability and altered tissue distribution was observed for paclitaxel, loperamide, vinblastine, ivermectin, cyclosporine A (CsA), human immunodeficiency virus (HIV) protease inhibitors, TBuMA and APM. Marked increases in accumulation of these drugs were observed in the brain, liver, intestine, and other tissues of the knockout versus wild-type animals. Oral administration of loperamide resulted in plasma levels that were two to three times higher in *mdr1a* knockout mice compared to wild-type mice. The lethal dose to wild-type mice was approximately 80 mg/kg, whereas, in the *mdr1a*-deficient mice, the lethal dose was 10 mg/kg. The knockout mice had clear central opiate effects, which were absent in the wild-type mice because of the low amount of this drug that normally crosses the blood-brain barrier. Similarly, a six-fold increase in the area under the plasma concentration versus time curve (AUC) and an 11-fold increase in C_{\max} for orally administered paclitaxel were observed in the *mdr1a*-deficient mice compared to the control animals. Consequently, the oral bioavailability of paclitaxel increased from 11% in wild-type mice to 35% in the knockout animals. Co-administration of the P-gp inhibitors PSC 833 or CsA with paclitaxel in wild-type animals resulted in a 10-fold increase in AUC, further supporting a role for P-gp in oral drug absorption. These data also clearly demonstrate the consequences of inhibition of P-gp on the pharmacokinetics of a co-administered drug. This increase is greater than that observed in the knockout mice and is likely due to inhibition of CYP3A in the intestine and liver, suggesting a combined role of P-gp and CYP3A in limiting oral bioavailability of substrate drugs.

Kim *et al.* (1998) recently observed that P-gp also limits the oral bioavailability of the HIV protease inhibitors indinavir, nelfinavir, and saquinavir, which suggests more effective treatment of this disease may be achieved by co-administration of a P-gp inhibitor with these agents. Administration of these protease inhibitors to *mdr1a*-deficient mice resulted in 2- to 5-fold

higher plasma concentrations and a 7- to 36-fold increased brain accumulation of the drugs. These authors suggest that targeted inhibition of P-gp would result in higher protease inhibitor concentrations and more effective therapy.

The Caco-2 human intestinal cell line is a well-studied model for assessing drug absorption and investigation of mechanisms that affect oral bioavailability. Hunter *et al.* (1993) used immunofluorescence with the MRK16 antibody to demonstrate apical expression of P-gp in these cells. Using specialized dualchamber tissue culture dishes, these authors observed transporter-mediated, directional, and saturable secretion of vinblastine from the basolateral toward the apical side of Caco-2 monolayers. Several P-gp modulators such as verapamil, MRK16, taxotere, 1,9-dideoxyforskolin, and nifedipine inhibited this transport. Furthermore, this inhibition led to a dose-dependent increase in vinblastine absorptive flux. Similarly, P-gp mediated time and concentration dependent polarized efflux of CsA was observed in Caco-2 cells and was suggested to be a key physiologic determinant of CsA oral bioavailability. These data provided early support for the hypothesis that P-gp, located at the tip of the intestinal villus, is a barrier for drug absorption.

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

Recent studies have demonstrated that interaction between intestinal drug metabolism by cytochrome P450 3A and P-gp-mediated transport may contribute to the poor oral bioavailability and high inter-patient and intra-patient variability in absorption of drugs. The liver has been classically viewed as the primary site of drug metabolism; recently, however, it has been recognized that a significant amount of drug metabolism occurs in the intestine and is mediated by CYP3A. Although the intestine does not quantitatively have as much CYP3A as the liver, the

enzyme is located in the differentiated villus cells, which are the site of drug absorption. Greater than 50% of clinically important drugs are metabolized by CYP3A; thus, its location in the intestine suggests a critical role for it in oral drug bioavailability. Recently, a striking overlap between the substrates for P-gp and cytochrome P450 3A family members has been observed. Simultaneous expression of these proteins in the intestine suggests complementary roles that may limit drug absorption and increase disposition. Another potential function for P-gp in the intestine may be to transport compounds back into the lumen. This would establish a cyclic pathway for drugs as they transit the intestine, thereby increasing the exposure time of drugs to drug-metabolizing enzymes (e.g., CYP3A) to act. The cooperative nature of CYP3A and P-gp presents a unique opportunity to affect substrate absorption and a significant potential for drug interaction (Silverman, 2000).

6. P-gp mediated drug-drug interactions

Drug-drug interactions mediated by inhibition and induction of P-gp have been reported in animals and humans. The pharmacokinetic consequences of P-gp inhibition and induction are similar to those observed for inhibition and induction of CYP enzymes. In other words, P-gp inhibition results in an increase in the systemic exposure and tissue distribution of drugs that are P-gp substrates, while P-gp induction leads to a decrease in the systemic exposure (Lin, 2003).

6.1 Drug interactions caused by P-gp inhibition

Because overlapping substrates between CYP3A4 and P-gp and many inhibitors can affect both CYP3A4 and P-gp, many drug interactions may involve both the enzyme and transporter systems. Therefore, it is important to differentiate the relative contribution of P-gp inhibition from that of CYP3A4 inhibition in order to make an appropriate interpretation of drug interaction data. For this reason, Wan del *et al.* (1999) have proposed using the ratio of the IC_{50} of CYP3A4 to the IC_{50} of P-gp as an index of the relative selectivity of a drug for P-gp inhibition versus CYP3A4 inhibition. If the ratio is much greater than unity, it means that the relative contribution by P-gp inhibition is quantitatively more significant. The relative selectivity is best exemplified by the following study by Choo *et al.* (2000). The IC_{50} values for ketonazole to inhibit digoxin transport and nifedipine metabolism were 1.4 and 0.15 μ M, respectively, and the corresponding values for LY-335979 were 0.024 and 5 μ M. Pretreatment with LY-335979

(25 mg/kg, i.v.) resulted in a 15-fold increase in brain levels of nelfinavir, but had little effect on plasma concentration in *mdr1a* (+/+) mice. On the other hand, co-administration of ketoconazole (50 mg/kg, i.v.) caused an 8-fold increase in plasma concentration in *mdr1a* (+/+) mice. From the values of IC₅₀ ratio (208 for LY-335979 and 0.13 for ketoconazole), it is clear that the increased brain levels of nelfinavir by LY-335979 are due exclusively to P-gp inhibition. In contrast, the increase in the brain concentrations of nelfinavir by ketoconazole is attributed mainly to the CYP3A inhibition.

P-gp inhibition as a cause of drug interaction has also been reported in humans. Perhaps the most compelling clinical evidence of P-gp-mediated drug interactions in humans is the interaction of digoxin with other cardiac drugs, such as verapamil and quinidine (Verschragen *et al.*, 1999; Bussey, 1982; Mordel *et al.*, 1993; Pedersen, 1985). A daily dose of 160 mg verapamil caused a 40% increase in digoxin plasma concentration, while a daily dose of 240 mg verapamil caused a 60-80% increase, suggesting dose-dependent P-gp inhibition. Because digoxin is exclusively eliminated in humans by renal excretion as unchanged drug, with minimal metabolism (Hinderling *et al.*, 1991), it is highly likely that the observed drug interaction between digoxin and verapamil (or quinidine) is due to inhibition of P-gp activity, resulting in increased absorption and decreased elimination of digoxin.

Ketoconazole is also a potent P-gp inhibitor. The IC₅₀ value of ketoconazole to inhibit digoxin transport in Caco-2 cells was determined to be 1.4 μ M (Choo *et al.*, 2000). Khaliq *et al.* (2000) studied the effect of ketoconazole on the blood brain barrier (BBB) penetration of ritonavir and saquinavir in HIV patients. Co-administration of ketoconazole (200 mg, p.o.) resulted in a marked increase in the CSF concentration of ritonavir and saquinavir, while ketoconazole had only a minimal effect on the plasma concentration of the drug. The ratio of CSF drug concentration to plasma unbound drug concentration for saquinavir and ritonavir was, respectively, increased from 0.06 and 0.09 without ketoconazole to 0.35 and 0.26 with ketoconazole. These results clearly demonstrated that ketoconazole increases the BBB penetration of saquinavir and ritonavir in HIV patients by inhibiting the P-gp transport.

Talinolol, a β -blocker, is eliminated from the body mainly by intestinal and renal excretion with minimal metabolism. In a clinical study, a P-gp-mediated interaction between talinolol and verapamil has been reported by Gramatte *et al.* (1999). The inhibitory effect of verapamil on the intestinal secretion of talinolol was determined in six healthy volunteers by using

the intestinal perfusion technique. While perfusing the small intestine with a verapamil-free solution, the mean intestinal secretion rate of talinolol was 4.0 $\mu\text{g}/\text{min}$ per 30-cm length of the intestine after an intravenous dose of talinolol. The intestinal secretion rate decreased to 2.0 $\mu\text{g}/\text{min}$ per 30-cm length when a verapamil containing solution (565 μM) was perfused. These results clearly suggested that the increased absorption of talinolol was partly attributed to the inhibition of intestinal P-gp function. Consistent with the clinical data, talinolol-verapamil interaction was observed in rats (Spahn-Langguth *et al.*, 1998). Co-administration of verapamil (4 mg/kg, p.o.) resulted in a 2.5- and 2.2-fold increase in the plasma oral AUC for *S*- and *R*-talinolol, respectively, after an oral dose of racemic talinolol in rats. However, after an intravenous dose of the racemic talinolol, the inhibitory effect of verapamil on talinolol was less significant and there was only a 40 and 30% increase in AUC, respectively, for *S*- and *R*-talinolol.

6.3 Drug interactions caused by P-gp induction

The most compelling evidence to date describing the P-gp induction as the cause of drug interactions was provided by Greiner *et al.* (1999) in a clinical study comparing the pharmacokinetics of digoxin before and during co-administration of rifampicin (600 mg/day for 10 days) in eight healthy volunteers. The plasma C_{max} and AUC of digoxin decreased from 5.4 ng/ml and 55 ng.h/ml before rifampicin pretreatment to 2.6 ng/ml and 38 ng.h/ml, respectively, during rifampicin pretreatment when the volunteers received a single dose of digoxin (1 mg). However, rifampicin pretreatment had little effect on the AUC and renal clearance of digoxin after intravenous administration of digoxin. Duodenal biopsies were obtained from each volunteer before and after administration of rifampicin. Rifampicin treatment increased intestinal P-gp content 3.5 fold, which correlated well with the oral AUC of digoxin in an inverse manner. Since digoxin is eliminated mainly by renal excretion and since digoxin is given orally at a very low dose, these results strongly suggest that digoxin-rifampicin interaction mainly occurs at the level of the intestine through P-gp-mediated induction. This means that the decreased plasma concentration of digoxin during rifampicin treatment is caused by a reduced bioavailability of digoxin as a result of induction of intestinal P-gp. Similarly, administration of St. John's Wort extract (three doses of 300 mg per day for 14 days) resulted in an 18% decrease of plasma AUC after a single digoxin dose 0.5 mg in healthy volunteers (Dürr *et al.*, 2000). Treatment with the extract resulted in a 1.4-fold increase in the expression of duodenal P-gp. The decreased plasma AUC correlated reasonably well with an increased intestinal P-gp expression.

The inductive effect of rifampicin on the pharmacokinetics of talinolol (a P-gp substrate), which is eliminated from the body predominantly by renal and intestinal excretion with minimal metabolism (<1.5%), has been studied in normal volunteers (Westphal *et al.*, 2000). The bioavailability and oral plasma AUC of talinolol were decreased from 55% and 873 ng.h/ml before rifampicin treatment to 35% and 565 ng.h/ml, respectively, during rifampicin co-administration (600 mg p.o., 9 days). During rifampicin treatment, total clearance of talinolol was increased significantly by 30% after intravenous administration of the drug. Treatment with rifampicin resulted in a significant increase in the expression of duodenal P-gp content by ~4-fold in these volunteers. The duodenal P-gp expression correlated significantly with the total clearance of talinolol. Since talinolol undergoes minimal metabolism, these results clearly demonstrated that the observed talinolol-rifampicin interaction is attributed mainly to a combination of a decrease in absorption and an increase in elimination as the result of P-gp induction.

Pretreatment with rifampicin also significantly decreased the systemic exposure of fexofenadine in healthy volunteers (Hamman *et al.*, 2001). Twenty volunteers received a 60 mg oral dose of fexofenadine before and after treatment with 600 mg of oral rifampicin for 6 days. The C_{max} and AUC of fexofenadine decreased by 2- and 3-fold, respectively, in volunteers after rifampicin treatment. The investigators concluded that the decreased plasma profiles were the result of a reduced bioavailability caused by induction of intestinal P-gp. This conclusion was based on an assumption that fexofenadine is not metabolized in humans. However, the assumption of the lack of metabolism may not be valid. From a human mass balance study, it was shown that approximately 80 and 11% of an oral dose of [14 C]fexofenadine were recovered in the feces and urine, respectively. Furthermore, MDL4,829, a metabolite of fexofenadine, accounted for ~20% of the radioactivity in urine (Lippert *et al.*, 1999). However, it is unknown if the fecal component represents unabsorbed drug or the result of biliary excretion, and information on the metabolites in feces is not available. Therefore, it is possible that the observed fexofenadine-rifampicin interaction is attributed to a combination of both CYP and P-gp induction. Furthermore, it demonstrated that organic anion transporting polypeptide (OATP) is involved in the hepatic uptake of fexofenadine (Cvetkovic *et al.*, 1999). It is possible than rifampicin is able to induce OATP. Thus, the involvement of OATP in the hepatic uptake and thereby biliary excretion of fexofenadine may further complicate the interpretation of the observed fexofenadine-rifampicin interaction.

Organic cation and anion transporter (OCT/OAT)

Secretion in the kidney of structurally diverse molecules including many drugs, environmental toxins and carcinogens is critical in the body's defense against foreign substances. The specificity of secretory pathways in the nephron for two distinct classes of substrates, organic anions and cations, was first described decades ago, and these pathways were well characterized using a variety of physiological techniques including isolated perfused nephrons and kidneys, micropuncture techniques, cell culture methods, and isolated renal plasma membrane vesicles. However, not until the mid-1990s were the molecular identities of the organic anion and cation transporters revealed. During the past decade, molecular studies have identified and characterized the renal transporters that play a role in drug elimination, toxicity, and response. Thus, we now can describe the overall secretory pathways for organic cations and their molecular and functional characteristics. Although the pharmacological focus is often on the kidney, there is useful information on the tissue distribution of these transporters. Molecular studies using site-directed mutagenesis have identified substrate-recognition and other functional domains of the transporters, and genetic studies of knockout mouse models have been used to characterize the physiological roles of individual transporters. Recently, studies have identified and functionally analyzed genetic polymorphisms and haplotypes of the relevant transporters in humans. Our understanding of organic anion transport has progressed in a similar fashion. In some cases, transporters that are considered organic anion or organic cation transporters have dual specificity for anions and cations (Giacomini and Sugiyama, 2006).

1. Organic cation transport

Structurally diverse organic cations are secreted in the proximal tubule. Many secreted organic cations are endogenous compounds (e.g., choline, N-methylnicotinamide, and dopamine), and renal secretion appears to be important in eliminating excess concentrations of these substances. However, the majority of drugs for therapeutic use including many antihistamine, antacids, antiarrhythmics, antihypertensives and anticholinergics are organic cations or weak bases, i.e. molecules with a transient or permanent positive net charge (Müller *et al.*, 2005) and toxin from the environment (e.g., nicotine). Organic cations that are secreted by the kidney may be either hydrophobic or hydrophilic (Giacomini and Sugiyama, 2006). The model for their secretion in the proximal tubule of the nephron is shown in Figure 9.

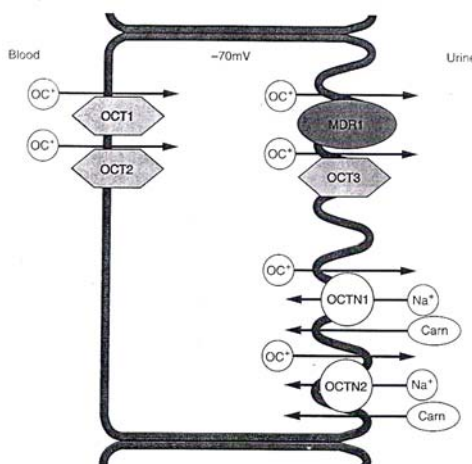


Figure 9 Model of organic secretory transporters in the proximal tubule

For the transepithelial flux of a compound (e.g., secretion), it is essential for the compound to traverse two membranes sequentially, the basolateral membrane facing the blood side and the apical membrane facing the tubular lumen. Distinct transporters on each membrane mediate each step of transport. Organic cations appear to cross the basolateral membrane by three distinct transporters in the solute carrier (SLC) family 22 (SLC22): OCT1 (SLC22A1), OCT2 (SLC22A2), and OCT3 (SLC22A3). Organic cations are transported across this membrane down their electrochemical gradient (-70 mV). Previous studies in isolated basolateral membrane vesicles demonstrate the presence of a potential-sensitive mechanism for organic cations. The cloned transporters OCT1, OCT2, and OCT3 are all potential sensitive and mechanistically coincide with previous studies of isolated basolateral membrane vesicles. Transport of organic cations from cell to tubular lumen cross the apical membrane occurs via an electroneutral proton-organic cation exchange mechanism in a variety of species, including human, dog, rabbit, and cat. Transporters assigned to the apical membrane are in the SLC22 family and termed novel organic cation transporters (OCTNs). In humans, these include OCTN1 (SLC22A4) and OCTN2 (SLC22A5). These bifunctional transporters are involved not only in organic cation secretion but also in carnitine reabsorption. In the reuptake mode, the transporters function as Na^+ cotransporters, relying on the inwardly driven Na^+ gradient created by $\text{Na}^+.\text{K}^-$ -ATPase to move carnitine from tubular lumen to cell. In the secretory mode, the transporters appear to function as proton-organic cation exchangers. That is, protons move from tubular lumen to cell interior in

exchange for organic cations, which move from cytosol to tubular lumen. The inwardly directed proton gradient (from tubular lumen to cytosol) is maintained by transporters in the SLC9 family (NHEs), which are Na^+/H^+ exchanges (antiporters). The bifunctional mechanism of OCTN1 and OCTN2 may not totally explain the organic cation-proton exchange mechanism that has been described in many studies in isolated plasma membrane vesicles. Of the two steps involved in secretory transport, transport across the luminal membrane appears to be rate-limiting (Giacomini and Sugiyama, 2006).

1.1 OCT1 (SLC22A1)

OCT1 was first cloned from a rat cDNA library. Subsequently orthologs were cloned from mouse, rabbit, and humans. Mammalian isoforms of OCT1, which vary in length from 554 to 556 amino acids, have 12 putative transmembrane domains and include several N-linked glycosylation sites. A long extracellular loop between transmembrane domains 1 and 2 is characteristic of the OCTs. The gene for the human OCT1 is mapped to chromosome 6 (6q26). In human, OCT1 is expressed primarily in the liver, with some expression in heart, intestine, and skeletal muscle. In mouse and rat, OCT1 is also abundant in the kidney, whereas in human, very modest levels of OCT1 mRNA transcripts are detected in kidney. The transport mechanism of OCT1 is electrogenic and saturable for transport of model small-molecular-weight organic cations including tetraethylammonium (TEA) and dopamine. Interestingly, OCT1 also can operate as an exchanger, mediating organic cation-organic cation exchange. That is, loading cells with organic cations such as unlabeled TEA can trans-stimulate the inward flux of organic cations such as MPP^+ . It also should be noted that organic cations can transinhibit OCT1. In particular, the hydrophobic organic cations quinine and quinidine, which are poor substrates of OCT1, when present on the cytosolic side of a membrane, can inhibit influx of organic cations via OCT1 (Giacomini and Sugiyama, 2006). The human OCT1 generally accepts a wide array of monovalent organic cations with molecular weights of less than 400 daltons, including many drug (e.g., procainamide, metformin, and pindolol) (Dresser *et al.*, 2001).

1.2 OCT2 (SLC22A2)

OCT2 was first cloned from a rat kidney cDNA library (Okuda *et al.*, 1996). Human, rabbit, mouse and pig orthologs all have been cloned. Mammalian orthologs range in length from 553 through 555 amino acids. Similar to OCT, OCT2 is predicted to have 12 transmembrane domains, including one N-linked glycosylation site. OCT2 is located adjacent

to OCT1 on chromosome 6 (6q26). Human, mouse, and rat orthologs of OCT2 are expressed in abundance in human kidney and to some extent in neuronal tissue such as choroids plexus. In the kidney, OCT2 is localized to the proximal tubule and to distal tubules and collecting ducts. In the proximal tubule, OCT2 is restricted to the basolateral membrane. OCT2 mammalian species orthologs are greater than 80% identical, whereas OCT1 and OCT2 paralogs are approximately 70% identical. The transport mechanism of OCT2 is similar to that of OCT1. In particular, OCT2-mediated transport of model organic cations MPP^+ and TEA is electrogenic, but like OCT1, OCT2 can support organic cation-organic cation exchange. Some studies show modest proton-organic cation exchange. More hydrophobic organic cations may inhibit OCT2 but may not be translocated by it. Like OCT1, OCT2 generally accepts a wide array of monovalent organic cations with molecular weights of less than 400 daltons. The apparent affinities of the human OCT1 and OCT2 paralogs for some organic cation substrates and inhibitors have been shown to be different in side-by-side comparison studies. Isoform-specific inhibitors of the OCTs are needed to determine the relative importance of OCT1 and OCT2 in the renal clearance of compounds in rodents, in which both isoforms are present in kidney. OCT2 is also present in neuronal tissues. However, studies with monoamine neurotransmitters demonstrate that dopamine, serotonin, histamine, and norepinephrine have low affinities for OCT2. These studies suggest that OCT2 may play a housekeeping role in neurons, taking up only excess concentrations of neurotransmitters. OCT2 also may be involved in recycling of neurotransmitters by taking up breakdown products, which in turn enter monoamine synthetic pathways (Giacomini and Sugiyama, 2006).

1.3 OCT3 (SLC22A3)

OCT3 was cloned initially from rat placenta (Kekuda *et al.*, 1998). Human and mouse orthologs have also been cloned. OCT3 consists of 551 amino acids and is predicted to have 12 transmembrane domains, including three N-linked glycosylation sites. hOCT3 is located in tandem with OCT1 and OCT2 on chromosome 6. Tissue distribution studies suggest that human OCT3 is expressed in liver, kidney, intestine, and placenta, although it appears to be expressed in considerably less abundance than OCT2 in the kidney. Like OCT1 and OCT2, it appears to have quantitative differences in its affinities for many organic cations. Some studies have suggested that OCT3 is the extraneuronal monoamine transporter based on its substrate specificity and potency of interaction with monoamine neurotransmitters. Because of its relatively

low abundance in the kidney, OCT3 may play only a limited role in renal drug elimination (Giacomini and Sugiyama, 2006).

1.4 OCTN1 (SLC22A4)

OCTN1 was cloned originally from human fetal liver, is expressed in the adult kidney, trachea, and bone marrow (Tamai *et al.*, 1997). The functional characteristics of OCTN1 suggest that it operates as an organic cation-proton exchanger. OCTN1-mediated influx of model organic cations is enhanced at alkaline pH, whereas efflux is increased by an inwardly directed proton gradient. OCTN1 contains a nucleotide-binding sequence motif, and transport of its substrates appears to be stimulated by cellular ATP content. OCTN1 also can function as an organic cation-organic cation exchanger. Although the subcellular localization of OCTN1 has not been demonstrated clearly, available data collectively suggest that OCTN1 functions as a bidirectional pH- and ATP-dependent transporter at the apical membrane in renal tubular epithelial cells (Giacomini and Sugiyama, 2006).

1.5 OCTN2 (SLC22A5)

OCTN2 was first cloned from human kidney and determined to be the transporter responsible for systemic carnitine deficiency (Tamai *et al.*, 1998). Rat OCTN2 mRNA is expressed predominantly in the cortex, with very little expression in the medulla, and is localized to the apical membrane of the proximal tubule. OCTN2 is a bifunctional transporter. That is, it transports L-carnitine with high affinity in an Na⁺ does not influence OCTN2 –mediated transport of organic cations such as TEA. Thus, OCTN2 is thought to function as both Na⁺-dependent carnitine transporter and Na⁺-independent organic cation transporter. Similarly, to OCTN1, OCTN2 transport of organic cations is sensitive to pH, suggesting that it may function as an organic cation exchanger. Studies in mice containing a missense mutation in *Slc22a5* suggest that organic cations are transported in a secretory direction by OCTN2, whereas carnitine is transported in a reabsorptive direction. Therefore, transport of L-carnitine by OCTN2 is Na⁺-dependent electrogenic process (Giacomini and Sugiyama, 2006).

2. Organic anion transport

As with organic cations (OCs), the proximal tubule is the primary site of renal organic anion (OA) secretion. Substrates for the pathways involved in renal OA transport include a diverse array of weak acids that have a net negative charge on carboxylate or sulfonyl residues

at physiological pH. Although a number of endogenous OAs have been shown to be actively secreted by the proximal tubule (e.g., 5'-hydroxyindoleacetic acid, riboflavin), it is generally accepted that the principal function of this process is clearing the body of xenobiotic agents, including many weakly acidic drugs (e.g., pravastatin, captopril, *p*-aminohippurate (PAH), and penicillins) and toxins (e.g., ochratoxin) (Wright and Dantzer, 2004; Giacomini and Sugiyama, 2006).

A current model for the transepithelial flux of organic anions in the proximal tubule is shown in figure 10. Two primary transporters on the basolateral membrane mediate the flux of organic anions from interstitial fluid to tubule cell: OAT (SLC22A6) and OAT3 (SLC22A8). Energetically, hydrophilic organic anions are transported across the basolateral membrane against an electrochemical gradient in exchange with intracellular α -ketoglutarate, which moves down its concentration gradient from cytosol to blood. The outwardly directed gradient of α -ketoglutarate is maintained at least in part by a basolateral Na^+ -dicarboxylate transporter (NaDC3). The Na^+ gradient that drives NaDC3 is maintained by Na^+, K^+ -ATPase (Giacomini and Sugiyama, 2006).

The mechanism responsible for the apical membrane transport of OA from tubule cell cytosol to tubular lumen remains controversial. Some studies suggested that OAT4 may serve as the luminal membrane transporter for organic anions. However, the movement of substrates via this transporter can be driven by exchange with α -ketoglutarate, suggested that OAT4 may function in the reabsorptive, rather than secretory, flux of organic anions. Other studies suggested that in the pig kidney, OATV1 serves as an electrogenic facilitated transporter on the apical membrane. The human ortholog of OATV1 is NPT1, or NaPi-1, originally cloned as a phosphate transporter. NPT1 can support the low-affinity transport across the apical membrane include MRP2 and MRP4, multidrug-resistance transporters in the ATP-binding cassette family C (ABCC). Both transporters interact with some organic anions and may actively pump their substrates from tubule cell cytosol to tubular lumen (Giacomini and Sugiyama, 2006).

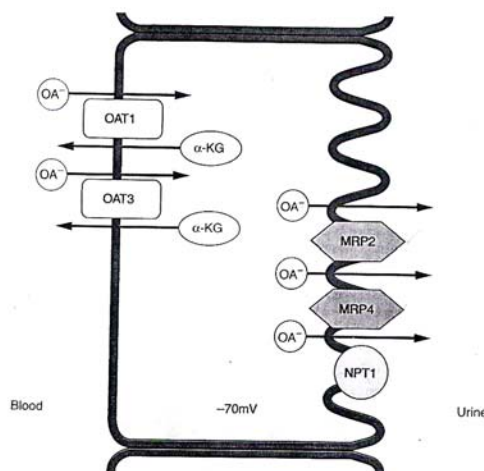


Figure 10 Model of organic anion secretory transporters in the proximal tubule

2.1 OAT1 (SLC22A6)

OAT1 was cloned from rat kidney. Mammalian isoforms of OAT1 vary in length from 545 to 551 amino acid. Immunohistochemical studies suggested that OAT1 is expressed on the basolateral membrane of the proximal tubule in human and rat. OAT1 exhibits saturable transport of organic anions such as PAH. This transport is tran-stimulated by other organic anions, including α -ketoglutarate. Thus, the inside negative-potential difference drives the efflux of the dicarboxylate α -ketoglutarate, which in turn, supports the influx of monocarboxylates such as PAH. Sex steroids also influence the level of expression of OAT1 mRNA. OAT1 generally transports small-molecular-weight organic anions that may be endogenous (e.g., PGE_2 and urate) or ingested drugs and toxins. Some neutral compounds are also transported by OAT1 at a lower affinity (e.g., cimetidine) (Wright and Dantzer, 2004).

2.2 OAT2 (SLC22A7)

OAT2 was cloned first from rat liver. This transporter has a gender-based tissue distribution between the liver and the kidney in rodents but not in humans, OAT2 is present in both kidney and liver. In the kidney, the transporter is localized to the basolateral membrane of the proximal tubule. Efforts to stimulate organic anion-organic anion exchange via OAT2 have not been successful, leading to the speculation that OAT2 is a basolateral membrane transporter that serves in the reabsorptive flux of organic anions from tubule cell cytosol to interstitial fluids. OAT2 transports many organic anions, including PAH, methotrexate, ochratoxin A, and glutarate.

Human, mouse, and rat orthologs of OAT2 have high affinities for the endogenous prostaglandin, PGE₂ (Giacomini and Sugiyama, 2006).

2.3 OAT3 (SLC22A8)

OAT3 was cloned from rat kidney. Two OAT3 orthologs have been cloned from human kidney. The second of these, hOAT3, codes for a 542-amino acid protein that supports transport of a wide range of OAs, including PAH, estrone sulfate, and methotrexate. In contrast, the first reported clone of human OAT3 encodes for a 568-amino acid protein. However, this longer cDNA does not appear to support transport. The distribution of OAT3 in humans differs from that in rats, with Northern blots showing expression to be virtually restricted to the kidney. Transported substrates include estrone sulfate (ES), indoxyl sulfate, PAH, ochratoxin A, PGE₂, benzylpenicillin, cephaloridine, and glutarate. Although it is evident that the selectivity of OAT3 overlaps that of OAT1 (and OAT2), affinities for several substrates appear to permit discrimination between OAT3 and these other transporters. ES has been frequently used as a test substrate in studies of OAT3 activity. OAT3 displays a moderately high affinity for ES, whereas OAT1 interacts little with ES. Like OAT1, OAT3 appears to be an exchanger that couples the outward flux of α -ketoglutarate to the inward flux of organic anions: The inside negative-potential difference repels α -ketoglutarate from the cells via OAT3, which in turn transports its substrates against a concentration gradient into the tubule cell cytosol (Wright and Dantzler, 2004).

2.4 OAT4 (SLC22A9)

OAT4 was cloned from a human renal cDNA library. Northern blot analysis indicated that OAT4 is most heavily expressed in the human kidney and placenta. Within the human kidney, OAT4 mRNA expression and is comparable to OAT2 expression. OAT4 appears to be localized to the luminal membrane of human proximal tubule cells (Wright and Dantzler, 2004). The previous study demonstrate that OA transport by OAT4 can be stimulated by transgradients of α -ketoglutarate, suggested that OAT4 may be involved in the reabsorption of OA from tubular lumen into cell. The specificity of OAT4 is narrow but includes ES and PAH (Giacomini and Sugiyama, 2006).

2.5 NPT1 (SLC17A1)

NPT1 was cloned originally from rabbit kidney, and subsequently cloned from rat, mouse, and human tissues. It is Na⁺-phosphate co-transporters. NPT1 is most expressed in the

luminal membrane of renal proximal tubules and is also expressed in liver and brain (Wright and Dantzler, 2004). NPT1 transports PAH, probenecid, and penicillin G. It appears to be part of the system involved in organic anion efflux from tubule cell to lumen (Giacomini and Sugiyama, 2006).

2.6 MRP2 (ABCC2)

MRP2 is expressed in the apical membrane of both human and rat renal proximal tubule cells, where it is thought to play a role in the efflux of organic anions into the tubular lumen. MRP2 supports the ATP-dependent export of a structurally diverse array of glutathione conjugates. There is also strong evidence supporting the contention that MRP2 mediates the ATP-dependent co-transport of glutathione with a number of nonconjugated substrates (Wright and Dantzler, 2004).

2.7 MRP4 (ABCC4)

MRP4 is expressed in the apical membrane of human renal proximal tubule cells, which immediately implicates it in the secretion of selected OAs. It transports a broad range of anionic conjugates, including glutathione, glucuronate, and phosphate conjugates (Wright and Dantzler, 2004). However, unlike MRP2, MRP4 appears to interact with various drugs, including methotrexate, cyclic nucleotide analogs, and antiviral nucleoside analogs. It is possible that MRP4 is involved in the apical flux of many drugs from cell to tubular lumen (Giacomini and Sugiyama, 2006).