CHAPTER 2

LITERATURE REVIEW

1. Introduction to Cytochromes P450

Drug metabolism can be classified as phase I and phase II reactions. In mammals, most xenobiotics are metabolised via hepatic phase I metabolism by means of CYP monooxygenases (Kohler *et al.*, 1997). Phase I reactions involve hydrolysis, reduction, and oxidation. NADPH is required as a coenzyme and O_2 is used as a substrate (Garrett and Grisham, 1995). These reactions expose or introduce a functional group (-OH, -NH_2, -SH or –COOH) into the drug molecule (Buck, 1997). A typical CYP catalysed reaction is as follow $NADPH + H^+ + O_2 + RH \longrightarrow NADP + H_2O$+R-OH. This reaction will transform hydrophobic compound into more water soluble compound that is and can be eliminated from the organism through urine or bile (Buck, 1997). Phase II reactions include glucuronidation, sulfation, acetylation, methylation and conjugation with glutathione (mercapturic acid synthesis) or with amino acids (such as glycine, taurine and glutamic acid) (Meyer and Rodvold, 1996). In phase I pathway, CYP is the most active among drug-metabolizing enzymes. This enzyme is also principally responsible for activation of procarcinogens and promutagens. Most clinically used drugs are metabolized by CYP.

CYP enzymes constitute a large superfamily of haem-containing monooxygenase involved in the metabolism of a wide variety of both exogenous and endogenous compounds (Degtyarenko *et al.*, 1995). They were first discovered in 1955 in rat liver microsomes and were also found in animals, plants, yeast and bacteria (Brodie *et al.*, 1958). These enzymes are embeded in the lipid bilayer of smooth endoplasmic reticulum (microsomes) of cells throughout the body, but the highest concentration was found in the liver (hepatocytes) and small intestine (Williams, 1962). Additionally, CYP are also found in the mitochondrial membrane (Williams, 1962). These enzymes are

responsible for the oxidative (Phase I) metabolism of a wide number of xenobiotics compounds, including several drugs and toxins (Buck, 1997). They biotransform lipophilic drugs to more polar compounds that can be advetually excreted by the kidney (Meyer and Rodvold, 1996). The metabolites are usually less active than the parent compounds, although certain drugs undergo biotransformation to pharmacologically active agents. In some cases the metabolites can be toxic, carcinogenic or teratogenic (Meyer and Rodvold, 1996).

The name "CYP" was derived from the fact that these proteins have a heme group. These enzymes are very opaque to standard spectroscopy, because they scatter light poorly. The only way to measure a spectrum of turbid samples like these was to make a special instrument with the light detector very close to the cuvette, and to use dual beams and do different spectroscopy (Joh *et al.,* 1995). In this way all the interfering substances and the light scattering could be subtracted out. With this setup, reduced microsomes with carbon monoxide (CO) gas added to one cuvette only give a very strong absorption band at 450 nm (Degtyarenko, 1995). This is called a reduced CO difference spectrum. The CO binds tightly to the ferrous heme, giving a difference between the absorbance of the two cuvettes. This spectrum was first observed in 1958 (Omura, 1964). Other heme containing proteins do not have this property. The reason why CYP absorbs in this range is the unusual ligand of the heme iron. Four ligands are provided by nitrogens on the heme ring (Degtyarenko, 1995). Above and below the plane of the heme, there is a room for two more ligands, the 5th and 6th ligands. For CYP, the 5th ligand is a thiolate anion, a sulfur with a negative charge, The sulfur comes from a conserved cysteine at the heme binding region of the active site (Smith *et al.*, 1991).

CYP may be called as mixed function monooxygenase because it adds an oxygen atom to numerous structurally-diverse substrates. A simplified scheme of oxidative cycle is presented in Figure 1. In the CYP catalytic cycle, the enzyme binds to its substrate (Step 1) and the heme ion is reduced from a valency of +3 to +2 by an electron transferred from NADPH via another flavoprotein called NADPH-P450 oxidoreductase

(Step 2). Then O_2 binds to the heme and is reduced by another electron (Step 3). A series of reaction occur that results in splitting of O_2 production of H_2O and oxidation of substrate (Step 4)(Correia, 1998). Drug oxidations of CYP are shown in Figure 1.

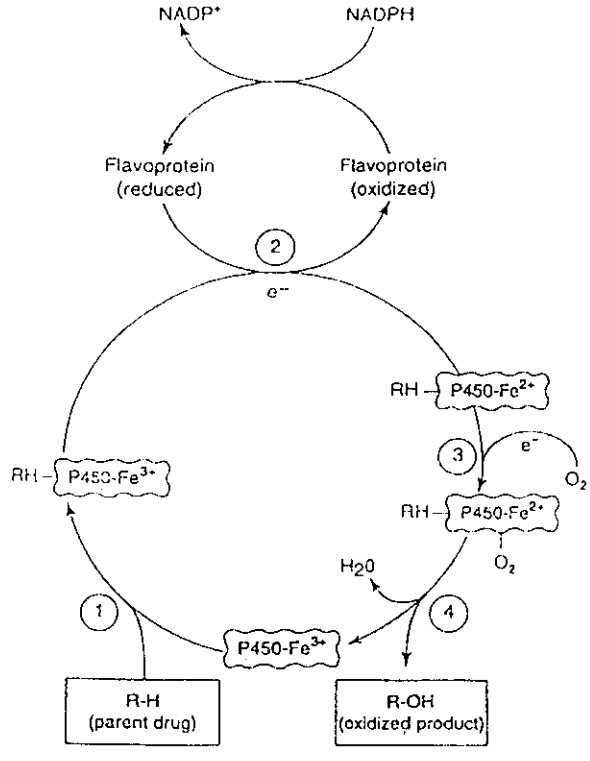

Figure 1 Cytochrome P450 cycle in drug oxidations (Correia, 1998)

There are two different kinds of electron transfer chains for CYP (Guengerich, 2000). These depend on the location of the enzyme in the cell. Some CYP are found in the mitochondrial inner membrane and some are found in the endoplasmic reticulum (ER) (Guengerich, 2000). Both types of CYP are membrane bound proteins. Mitochondrial P450 systems have three compounds; a FAD containing flavoprotein (NADPH or NADH-dependent reductase), an iron-sulfur protein and CYP. The eukaryotic microsomal P450 system contains two components: NADPH:P450 reductase, a flavoprotein containing both FAD and FMN, and CYP (Smith *et al.*, 1991).

All the CYP containing monooxygenase systems described so far share common structural and functional domain architecture (Degtyarenko, 1995). The term "domain" is

a polypeptide existing as an independently folding unit and possessing a certain function. All the P450 systems can be consisted of three-domain systems (Smith *et al.*, 1991) as follow: NADH- or NADPH-dependent FAD containing reductase (FAD domain); an iron-sulfur protein (in a three-component system) or FMN-binding domain (in a two- and one component system) and CYP domain (heme domain)

2. Human CYPs

Human CYPs are involved in the metabolism of xenobiotic (drugs and carcinogens) and endogenous compound such as steroids, fatty acid and other important lipids e.g. prostacyclins and thromboxane A2 (Shimada *et al.*, 1994). The CYP enzymes play an important role in phase I metabolism of many drugs. The broad range of drugs that undergo CYP mediated oxidative biotransformations is responsible for the large number of clinically significant drug interactions during multiple drug therapy (Badyal and Dadhich, 2001). . Many studies have shown effect of liver disease on CYP enzymes. In cirrhotic patients, expression of 1A2, 2E1 and 3A isoenzymes is decreased. There are reports of alteration of clearance of drugs metabolized by 3A4 in cirrhosis patients (Murray, 1992). Activity of CYP 2C19 was reported to be decreased in patients with liver disease, but activity of 2D6 was unaltered (Adedóyin *et al.*, 1998). Levels of 2C subfamily have been reported to be upregulated in patients with hepatic carcinoma (Murray, 1992). Detailed knowledge of these isoenzymes affected in disease states would be used to enhance the design of rational drug therapy.

2.1 Classification of human CYPs

There are now more than 2,400 CYP sequences known and in human these gene can be group in to 18 families of CYP genes and 43 subfamilies (Flockhart, *et al.*, 1994). At least 53 different CYP genes and 24 pseudogenes have been identified in human (Table1). The three main CYP genes family, CYP1, CYP2 and CYP3 are responsible for the vast majority of drugs metabolism and account for at least 70% of the total CYP content in human liver samples. Although the, CYP1, and CYP3 gene families are relatively simple (i.e. CYP1A and CYP3A) the CYP2 gene families is comprised of many subfamies (e.g., CYP2A, CYP2B, CYP2C, CYP2D, CYP2E, etc). These isoforms have the

same oxidizing center (the heme iron), but different by their protein structures (Lin and Lu, 1998).

CYP enzymes are divided into families based on amino acid sequence similarities and each family can be further separated into subfamilies which are designated by capital letters following the family designation (e.g., CYP3A) (Smith, 1991). Individual enzymes are subsequently indicated by arabic numerals (e.g., CYP3A4). An enzyme belongs to the same family when the amino acid sequence possesses more than 40% homology, enzymes with more than 55% homology belong to a subfamily and individual enzymes can have up to 97% homology between the sequences (Vercruysse, 1997). An individual CYP enzyme may be able to metabolize many different drugs and a given drug may be primarily metabolized by a single enzyme (Meyer and Rodvold, 1996).

Table 1. Human CYP families, gene and their subfamilies

CYP family	genes and their subfamilies
CYP1	3 subfamilies, 3 genes, 1 pseudogene
CYP2	13 subfamilies, 16 genes, 16 pseudogenes
CYP3	1 subfamily, 4 genes, 2 pseudogenes
CYP4	5 subfamilies, 11 genes, 10 pseudogenes)
CYP5	1 subfamily, 1 gene
CYP7	2 subfamily
CYP8	2 subfamily
CYP11	2 subfamily, 3 gene
CYP17	1 subfamily, 1 gene
CYP19	1 subfamily, 1 gene
CYP21	1 subfamily, 1 gene, 1 pseudogene
CYP24	1 subfamily, 1 gene
CYP26	3 subfamily
CYP27	3 subfamily
CYP39	1 subfamily
CYP46	1 subfamily
CYP51	1 subfamily, 1 gene, 3 pseudogenes

Sources : Nelson *et al.*, 1996

2.2 Function of CYPs

CYPs play comon roles in the bioactivation and detoxication of a wide variety of xenobiotic substances including drugs and carcinogens as well as synthesis and metabolism of endogenous compounds (Table 2). But less common role of CYP in drug metabolism is the catalyzed oxidation of drugs to the active metabolite (Guengerich, 2000). The enzymes belong to the families CYP1, CYP2 and CYP3 catalyze the oxidative biotransformation of exogenous compounds, including many drugs, (pro) carcinogens, (pro) mutagens and alcohols. The other CYP families invole in the metabolism of endogenous compounds including fatty acid, steroid hormones and prostaglandins (Table 2). CYP metabolise lipophilic to hydrophilic compounds, which can be excreated by the kidney (Jan and Linda, 1999). CYP catalysis will lower the plasma concentration of a drug and also the concentration of the drug at the target site (Guengerich, 2000). The human hepatic CYP system consists of over 30 related isoenzymes with different, sometimes overlaping, substrate specificities (Jan and Linda, 1999).

Table 2. Human CYP families and their main functions

CYP family	Main functions
CYP1	Xenobiotic metabolism
CYP2	Xenobiotic metabolism
CYP3	Xenobiotic and steroid metabolism
CYP4	Fatty acid hydroxylation
CYP5	Thromboxane synthesis
CYP7	Cholesterol 7α-hydroxylation
CYP8	Prostacyclin synthesis
CYP11	Cholesterol side-chain cleavage Steroid 11 α-hydroxylation, Aldosterone synthesis
CYP17	Steroid 17α-hydroxylation
CYP19	Androgen aromatization
CYP21	Steroid 21-hydroxylation
CYP24	Steroid 24-hydroxylation
CYP26	Retinoic acid hydroxylation
CYP27	Steroid 27-hydroxylation
CYP39	Unknown
CYP46	Cholesterol 24-hydroxylation
CYP51	Sterol biosynthesis

Sources : Nelson *et al.*,1996

2.3 Inhibition of CYP enzymes

Enzyme inhibition generally involves competition with another drug for enzyme binding sites, and usually begins with the first dose of the inhibitor. Duration of inhibition corresponds to the half-lives of the respective drugs. The mechanism of CYP inhibition can be divided grossly into three categories: reversible inhibitions, quasi-irreversible inhibitions and irreversible inhibitions. Among these, reversible inhibitions is probably the most common mechanism responsible for the documented drug interactions (Lin and Lu, 1998; Halpert, 1995).

2.3.1 Reversible Inhibition

Many of the potent reversible CYP inhibitors are nitrogen-containing drugs, including imidazole, pyridines and quinolines (Murry, 1992). These compounds cannot only bind to the prosthetic heme iron, but also to the lipophilic region of the protein. Inhibitor that simultanously binds to both regions is inherently more potent inhibitor. Reversible Inhibition can be competitive or non-competitive (Badyal and Dadhich, 2001). A drug may inhibit the CYP isoenzyme whether or not it is a substrate for that isoenzyme. If the two drugs are substrates for the same CYP isoenzyme then metabolism of one or both the drug may be delayed (Lin and lu, 1998). Erythromycin and midazolam both are substrates for 3A4 isoenzyme so, there is competition for enzyme sites and metabolism of midazolam is inhibited (Olkkola *et al.*, 1994). These drugs are converted through multiple CYP dependent steps to nitroso derivatives that bind with high affinity to the reduced form of CYP enzymes. Thus CYP enzymes are unavailable for further oxidation and synthesis of new enzymes is therefore the only means by which activity can be restored and this may take several days (Murray, 1992). Cimetidine and macrolide antimicrobials directly form a complex with heme moiety of CYP isoenzyme (Murray, 1992). Cimetidine, amiodarone and stiripentol are non-specific inhibitors of CYP450 enzyme system (Murray, 1992).

2.3.2 Irreversible Inhibition of CYP

Drugs containing functional groups can be oxidized by CYP to reactive intermediates that cause irreversible inactivation of the enzyme prior to its release from the active site (Murray, 1992). Because metabolic activation is required for enzyme inactivation, these drugs are classified as mechanism-based inactivators or suicide substrates (Guengerich, 2000). The mechanism-based inactivation of CYP may result from irreversible alteration of heme or protein, or a combination of both (Murray, 1992). In general, modification of the heme 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 tranfer and oxygen activation, are modified (Murray, 1992). Several α-ethinyl substituted steroids e.g. ethinylestradiol, gestodene

and levonorgestrel are reported to cause machanism-based inhibition (Guengerich, 2000).

2.3.2.1 Heme Alkylation

Drugs containing terminal double-bond (olefins) or triplebond (acetylenes) can be oxidized by CYP to radical intermediates that alkylate the prosthetic heme group and inactivate the enzyme (Collman *et al.*, 1985). The evidence for heme alkylation includes the demonstration of equimolar loss of enzyme and heme, as well as the isolation and structural characterization of the heme adducts (Collman *et al.*, 1985). Heme alkylation is initiated by the addition of activated oxygen to the internal carbon of the double or triple bond and is terminate by binding to heme pyrrole nitrogen (Correia, 1998). It is interesting to note that linear acetylenes react with the nitrogen of pyrrole ring A of CYP2B1 in liver microsomes of phenobarbital–induced rat, whereas linear olefins react with the nitrogen of pyrrole ring D (Correia, 1998).

2.3.2.2 Covalent Binding to Apoprotein

The best known example of inactivation of CYP through protein modification by a suicide inactivator is that of chloramphenicol (Halpert *et al.*, 1985). The dichloroacetamido group is an oxamyl moiety that acylates a lysine residual in the CYP active center (Halpert *et al.*, 1985). This acylation event interferes with the transfer of electrons from CYP reductase to the heme group of CYP and thereby prevents catalytic turnover of the enzyme. The inactivation by chloramphenicol is not uniform for all CYPs. Studies with rat liver microsomes revealed that CYP2B1, CYP2C6 and CYP2C are susceptible to inactivation by chloramphenicol, whereas CYP1A1 and CYP1A2 are resistant.

Although terminal acetylenes have been known to alkylate the prosthetic heme group, some terminal acetylene compounds, such as 2-ethylnylnaphalene, inactivate CYP by binding covalently to the protein with little loss of the heme group. 2-ethylnylnapthalene is converted by CYP2B1 to a ketene, which modified an active site peptide that includes Thr-302, a highly conserved residue known to play role in oxygen activation.

Oxidation of sulfur groups in drug molecules can result in the modification of the CYP protein. A variety of sulfur compounds inactivate CYP by binding covalently to protein after the enzyme oxidatively activates them. CYP inactivation by sulfur compound is believed to be involved with sulfur oxidation that generates reactive sulphur metabolites. Tienilic acid, a substituted thiophene, is oxidized yeast-expressed human CYP2C9 to a reactive metabolite, presumably a thiophene sulfuroxide that binds covalently to the CYP apoprotein.

The protein modification is caused by formation of a sulfur reactive metabolite, rather than formation of hydrodisulfides (Halpert *et al.*, 1985). Althogh covalent binding of the protein can be partially prevented by glutathione, the activity of the enzyme inactivated by tienilic acid cannot be restored by glutathione. In addition, diallyl sulfide, a flavour component of garlic, is known to be a potent suicide inhibitor of CYP2E1. The mechanism which diallyl sulfide inhibits CYP2E1 involves initial oxidation at sulfur to give diallyl sulfone which then undergoes metabolic activation on 1 or other terminal olefin groups to produce the ultimate reactive species (Lin and Lu, 1998; Halpert, 1995). Inhibitors of some CYP are demonstrated in Table 3.

Table 3. Inhibitors of some CYP (Melanie *et al.*, 1985).

1A2	2B6	2C19	2C9	2D6
amiodarone	thiotepa	cimetidine	amiodarone	amiodarone
cimetidine	ticlopidine	felbamate	fluconazole	fluconazole
fluoroquinolones		fluoxetine	fluvastatin	fluvastatin
fluvoxamine		fluvoxamine	fluvoxamine	fluvoxamine
furafylline		indomethacin	isoniazid	isoniazid
interferon		ketoconazole	lovastatin	lovastatin
methoxsalen		lansoprazole	paroxetine	paroxetine
mibefradil		modafinil	phenylbutazone	phenylbutazone
ticlopidine		omeprazole	probenecid	probenecid
		paroxetine	sertraline	sertraline
		probenecid	sulfaphenazole	sulfaphenazole
		ticlopidine	teniposide	teniposide
		topiramate	trimethoprim	trimethoprim

2) Peroxisome proliferator activated receptor (PPAR) that drugs act through a binding protein called the "PPAR" (Guengerich, 2000).

3) Retinoid X receptor (RXR) is receptor that drug is bound to this protein. It migrates to the nucleus heterodimerizes with RXR and binds to specific DNA sequences in the regulatory region of genes that are needed for peroxisome generation for example induction by rifampicin (Degtyarenko, 1995). Evidence from clinical drug interaction and phenotyping studies suggests that the CYP2C19 gene is inducible by at least two drugs *in vivo* in human, rifampin and artemisinin (Degtyarenko, 1995).

4) Constitutive androstane receptor (CAR) involved in induction by phenobarbital. Inducers of CYP are summarized in Table 4.

Table 4 Inducers of CYP and drug interactions

CYP	Inducer	Drugs affected	References
1A2	Tobacco	Theophylline	Sarkar and Duchin,1988
2C9	Rifampicin	Phenytoin	Levy,1999
2C19	Artemisinin	Omeprazole	Svensen et al., 1998
	Rifampicin	Phenytoin, Hexobarbital	Zhou et al., 1990, Zilly et al., 1975
2E1	Ethanol	Acetaminophen	Raucy et al., 1989
3A4	Rifampicin	Protease inhibitors	Murray,1992
		Diazepam, triazolam and Midazolam	Villika et al., 1997
		Estradiol, norgestrel	Crovo et al., 1999
		Ondansetron	Villikka et al., 1999
	Carbamazepine	Itraconazole	Bonay et al., 1993
		Vincristine	Villikka et al., 1999
		Protease inhibitors	Murray,1992
		Midazolam	Villika et al., 1997
	Phenytoin and	Midazolam	Villika et al., 1997
	Phenobarbitone	Vincristine	Villikka et al.,1999
		Carbamazepine	Badyal and

3. CYP2C19

CYP2C19 is the one of genes human CYP2C subfamily. The human CYP2C subfamily contains four highly homologous genes: 2C8, 2C9, 2C18 and 2C19. CYP2C19 is a protein of 490 amino acids. CYP2C19 gene has 9 exon 1473 base pairs (Romkes *et al.*, 1991) and is located in a cluster on chromosome 10 (Nelson *et al.*, 1996). It is mainly present in the liver but a significant activity has been identified in the gut wall. Interestingly, the splicing of CYP2C mRNA transcripts has been shown to produce chimeric mRNAs containing exons from several CYP2C genes (Romkes *et al.*, 1991). The biological function of these mRNAs is unknown. CYP2C accounts for about 20% of

the total human liver CYP content (Shimada *et al.*, 1994). CYP2C9 is the main CYP2C in human liver, followed by CYP2C8 and CYP2C19 (Goldstein and de Morais, 1994). CYP2C mRNA and protein are induced in primary hepatocytes by phenobarbital and rifampicin (Crovo *et al.*, 1999). Rifampicin and barbiturates can induce CYP2C proteins and related activities in vivo (Feng *et al.*, 1999). Pharmaceutical substrates for CYP2C include diazepam, omeprazole, mephenytoin, tolbutamide, and warfarin as well as many non-steroidal anti-inflammatory drugs (Gotoh, 1992).). Selective substrates include taxol for CYP2C8, tolbutamide for CYP2C9, and mephenytoin for CYP2C19 (Gotoh, 1992). The CYP2C19 poor metabolizer phenotype is detected in 2-5% of Caucasians and in about 13-23% of Asians (de Morais *et al.*, 1994a, 1994b; Balain *et al.*, 1995; Xiao *et al.*, 1997; Ibeanu *et al.*, 1998).

4. Substrates of CYP2C19

The substrates of CYP2C19 may be devided into three categories based on the contribution of CYP2C19 to the overall oral clearance of the drug: ≥80% (e.g omeprazole, lansoprazole, pantoprazole, (*S*)-mephenytoin, (*R*)-mephobarbital, (*R*)-hexobarbital and carisoprodol), 30-65% (e.g proguanil, imipramine, clomipramine, moclobemide, diazepam, flunitrazepam, citalopram and fluoxetine) and <30% (e.g. phenytoin, propranolol) (Desta *et al.*, 2002).

4.1 Mephenytoin

Mephenytoin was introduced in 1945 as an anticonvulsant agent, and since then it has been shown to be effective in the control of grand mal, focal, Jacksonian and psychomotor seizures, but limited usefulness for petit mal attacks (Kalow, 1986). It is clinically available as a racemic mixture of *R*- and *S*-enantiomers, and its metabolism is stereospecific in man (Küpfer and Presig, 1984). The *R*-enantiomers is slowly metabolized by oxidative demethylation to phenylethylhydantoin (PEH or nirvanol), while the *S*-enantiomers is rapidly hydroxylated to 4-hydroxymephenytoin. The aromatic *S*-hydroxylation is the stereoselective step in the metabolism and is impaired in poor metabolizers (PMs) (Wedlund *et al.*, 1985). PM phenotype, characterized by an impaired

ability to metabolize *S*-mephenytoin via *S*-mephenytoin hydroxylase, represents an autosomal recessive trait (Inaba *et al.*, 1984; Ward *et al.*, 1989). The distribution of the metabolic capacity of *S*-mephenytoin in a population showed a clear-cut bimodallity and was grouped into the two distinct phenotypes (PMs and EMs) (Wedlund *et al.*, 1984).

Since the stereospecific metabolic rate of racemic mephenytoin after the oral dose to individuals with it impaired 4-hydroxylation could be identified from the urinary metabolic profile, the genetic trait have been assessed after a test dose (100 or 50 mg) of racemic mephenytoin as a tablet (Mesantoin, Sandoz), with the postdose 8- to 24-hour urine collection (Wedlund *et al.*, 1984). The respective phenotypes are assigned either on the basis of the amount of 4-hydroxymephenytoin recovered in urine (high recovery in EMs, low recovery in PMs) (Küpfer and Presig, 1984), or on the enantiomeric ratio of S:R mephenytoin ($\geq$ 0.8 in PMs) (Wedlund *et al.*, 1984). However, *S/R* ratio need to be reanalysed after acidic hydrolysis before assigning them to the PM category (Zhang *et al.*,1992). The S/R will increase in EM, but not in PM, because of hydrolysis of a conjugate of S-mephenytoin excreted in EM, but not in PM (Wedlund *et al.*,1987; Tybring and Bertilsson, 1992). The metabolism pathway of mephenytoin in human are shown in Figure 2.

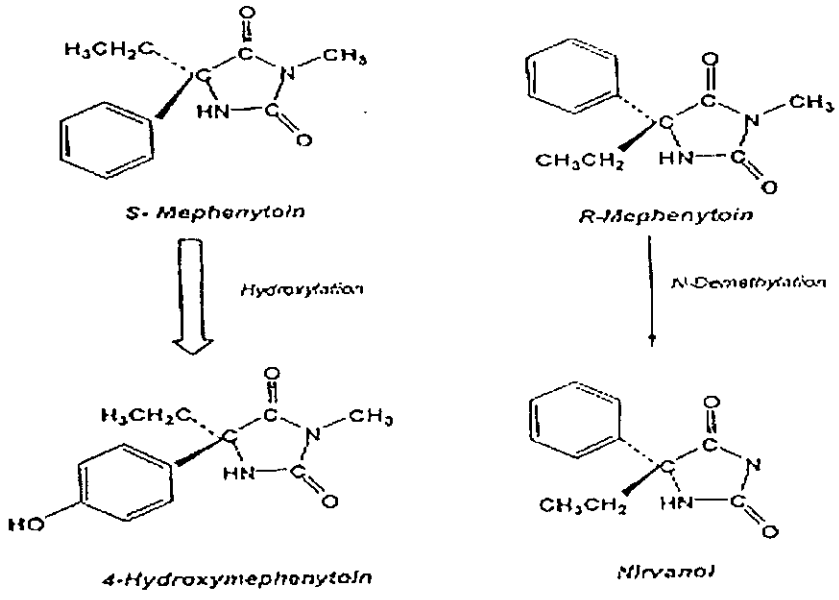

Figure 2 Metabolic pathway of mephenytoin in human (Goldstein *et al.*, 1994).

The 4'-hydroxylation of the *S*-enantiomer of mephenytoin has traditionally been used to separate poor and extensive metabolizers phenotypes of CYP2C19. However mephenytoin is not an ideal probe drug because side effect of a single dose of mephenytoin, principally sedation, limit its use in poor metabolizers, the elderly, children, and individuals with low body weight (Setiabudy *et al.*, 1992). Recently, omeprazole has been shown to be a useful probe for measuring the activity of CYP2C19.

4.2 Omeprazole and other proton pump inhibitors

Omeprazole (5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl] sulphinyl]-1*H*-benzimidazole) is a pyridinylsulfinylbenzimidazole compound, a agent referred to as proton pump inhibitor (Andersson *et al.*, 1993). It acts on the H^+/K^+ adenosine triphosphatase to regulate acid production in the gastric mucosa of stomach and is used to treat various acid-related gastrointestinal disorders. Its therapeutic potential has been documented as a potent long-acting inhibitor of gastric acid secretion for use in the treatment of various hyperacidic or hypersecretory conditions includings Zollinger-Ellison syndrome (Andersson *et al.*, 1992). Omeprazole is generally used in combination with antibacterial treatments to eradicate *H. pylori* infections, which are closely associated

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with peptic ulcer. Omeprazole is metabolised by CYP2C19 and CYP3A4 to two major plasma metabolites, 5'-hydroxyomeprazole and omeprazole sulphone, respectively. The affinity of omeprazole for CYP2C19 is known to be approximately 10 times greater than its affinity for CYP3A4 (Meyer, 1996). The metabolic pathways of omeprazole in human are shown in Figure 3.

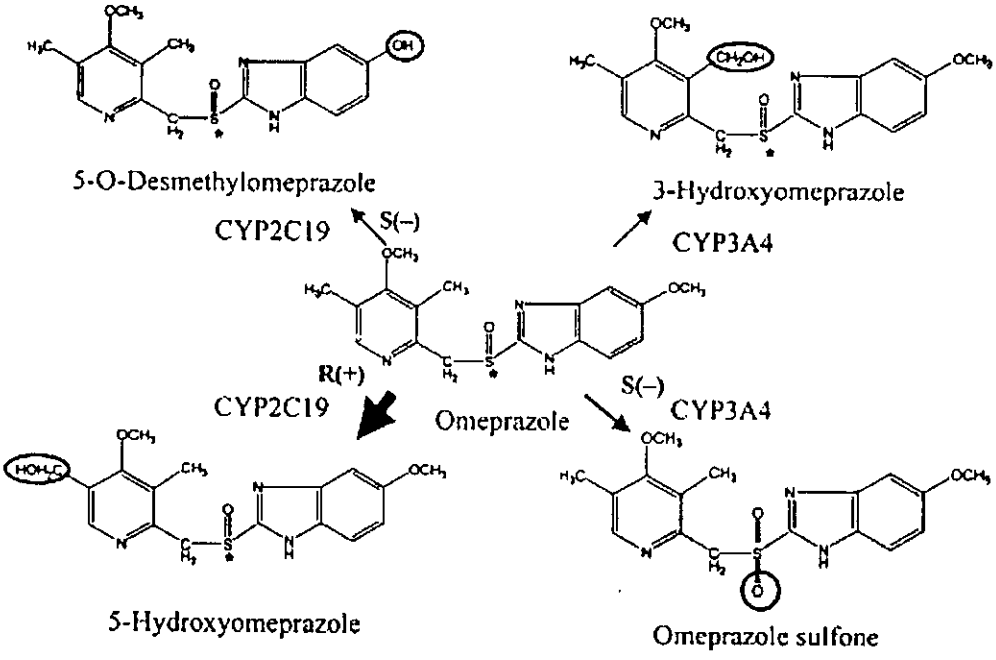

Figure 3 Metabolic pathway of omeprazole in human (Andersson *et al.*, 1993)

The metabolic ratio of omeprazole to 5'-hydroxyomeprazole in plasma 2 to 3 hours after a single oral dose of 20 mg omeprazole is significantly correlated with the *S/R* mephenytoin ratio (Andersson *et al.*, 1992; Chang *et al.*, 1995) and with the CYP2C19 genotype status (Chang *et al.*, 1995). Omeprazole has been used as a probe for measuring the activity of CYP2C19 in vivo in many studies (Chang *et al.*, 1995).

It has been shown that a higher concentration of omeprazole in poor metabolizers results in greater gastric acid suppression as compared with extensive metabolizers (Chang *et al.*, 1995). Heterozygous genotype (*CYP2C19*1/*2*) in Chinese extensive

metabolizers demonstrated a 28% decrease in the metabolism of omeprazole as compared with homozygous genotype (*CYP2C19*1/*1*) extensive metabolizers (Andersson *et al.*, 1992). Similars observation were made in the metabolism of omeprazole in Japanese subjects (Kubota *et al.*, 1996).

Omeprazole concentrations in plasma show a pronounced interindividual variability with administering the same doses (Anderson *et al.*, 1992). Cure rates for *H. pylori* in patients receiving omeprazole and amoxicillin were found to be 28% in homozygous extensive metabolizers (*CYP2C19*1/*1*), 60% in heterozygous extensive metabolizers (*CYP2C19*1/*2* and *CYP2C19*1/*3*), and 100% in poor metabolizers (*CYP2C19*2/*2* and *CYP2C19*2/*3*) (Furuta *et al.*, 1998). This result sugested that the dose of omeprazole should be adjusted in extensive metabolizers.

The hydroxylation index (HI) which is proportion concentrations of omeprazole and 5-hydroxyomeprazole is used for phenotyping of the CYP2C19 polymorphism. The subjects take 20 mg of omeprazole and then after 3 hours, the plasma concentrations of omeprazole and 5-hydroxyomeprazole are measured. Individuals with the HI higher than 7 are categorized as PMs, whereas the HI lower than 7.0 are classified as EMs (Andersson *et al.*, 1992). There are several other proton pump inhibitors including pantoprazole, rabeprazole and lansoprazole whose metabolism has also been shown to be dependent on CYP2C19 (Furuta *et al.*, 2001). For example, pantoprazole has a 6-fold increase in its plasma AUC, and a 5-fold shorter half-life in EMs of mephenytoin than in PMs (Furuta *et al.*, 1999). Pantoprazole lacks the 5-methyl group on the pyridine ring of omeprazole which is hydroxylated by CYP2C19. However, the demethylation of the 4-position of the pyridine ring is affected in CYP2C19 PMs (Shirai *et al.*, 2002). Lansoprazole is structurally related to omeprazole and its 5-hydroxylation is mediated by CYP2C19 (Andersson, 1993). The oral clearance of lansoprazole is about 6.5 times lower in oral clearance of mephenytion than EMs, and the AUC is also greater (Sohn *et al.*, 1997). These data indicate that metabolism of lansoprazole is highly dependent on the CYP2C19 genotype.

4.3 Chloroguanide (Proguanil)

Chloroguanide (Proguanil) is an arylbiguanide and was introduced as an antimalarial agent in 1940s. It has been used as a useful prophylactic agent against malarial infections in endemic areas. This drug has been used in combination chemotherapy with other antimalarial drugs such as chloroquine, atovaquone, sulfonamides, quinine or dapsone. Since the emergence and spread of multi drug-resistant *Plasmodium falciparum* the choice of the drug to prevent malarial infections has become problematic (Ward, 1991). In various areas endemic with *Plasmodium falciparum* malaria, chloroquine is no longer the drug of choice (Edstein *et al.*, 1986).

CYP2C19 and CYP3A4 accounted for about 73% and 16% of conversion of proguanil to cycloguanil in human liver microsomes (Coller *et al.*, 1997). The formation of cycloguanil cosegregates with PMs of mephenytoin, and the proguanil/cycloguanil ratio tends to be higher but the demarcation of the antimode is not clear (Brosen *et al.*, 1995). Proguanil may be a suitable phenotyping probe for the CYP2C19 genetic polymorphism, however the exact antimode of the urinary metabolic ratio chosen to separate poor and extensive metabolizers needs further investigation (Coller *et al.*, 1997). The antimalarial activity exvivo of plasma samples obtained from Thai EMs and PMs given proguanil was studies using isolated *P. falciparum* (Edstein *et al.*, 1996). There are clear difference in pharmacokinetic parameters between EM and PM individuals but no difference was observed with respect to the antimalarial effect of the proguanil *in vitro* between the groups (Edstein *et al.*, 1996). Therefore, the proguanil/cycloguanil ratio is not a good probe for the CYP2C19 polymorphism.

4.4 Diazepam

Diazepam is extensively metabolized in the liver through CYP-catalyzed reactions, that is about 60% of a given dose is *N*-demethylated to produce a major metabolite *N*-desmethyldiazepam (Bertisson *et al.*, 1989) and the remaining is converted to temazepam though C_3-hydroxylation (Qin *et al.*, 1999). CYP2C19 is a major enzyme involved in the *N*-demethylation of diazepam at low substrate concentrations (Wan *et al.*, 1996). There is a good correlation between the levels of CYP2C19 activity and the

formation of *N*-desmethyldiazepam after administration of diazepam (Bertisson *et al.*, 1989; Sohn *et al.*, 1992). The study in healthy Han Chinese subjects with different CYP2C19 genotypes demonstrated the gene dosage of CYP2C19 can markedly affects the metabolism and disposition of diazepam (Qin *et al.*, 1999). The plasma elimination half-life of diazepam in *CYP2C19*2/*2* subjects issignificantly longer than those of heterozygous *CYP2C19*1/*2* or those of heterozygous *CYP2C19*1/*3* (Qin *et al.*, 1999).

4.5 Tricyclic antidepressants (TCAs)

TCAs include amitriptyline, imipramine, nortriptyline and desipramine are strongly and competitively inhibited phenytoin p-hydroxylation in microsomal incubations. In contrast, nortriptyline and desipramine produced only weak inhibition. TCAs inhibit both CYP2D6 and CYP2C19. Interaction between TCAs and phenytoin involves inhibition of CYP2C19-catalyzed phenytoin p-hydroxylation (Shin *et al.*, 1992). The relationship between the genetic polymorphism of S-mephenytoin 4'-hydroxylation catalyzed by CYP2C19 and the N-demethylation of imipramine was examined in 10 Japanese depressed patients. The N-demethylation of imipramine is impaired in patients with genetic defects in the CYP2C19 gene. Genotype determination may be useful in preventing side effects induced by unexpectedly elevated levels of imipramine.

4.6 Phenytoin

CYP2C19 may be also involved in the metabolism of phenytoin (PHT). A population pharmacokinetic analysis of PHT was performed in Japanese adult patients with epilepsy who have different genotypes of CYP2C9 (Arg144/Cys, Ile359/Leu), *CYP2C19*1, CYP2C19*2, CYP2C19*3* and their serum concentrations of 5-(4-hydroxyphenyl)-5-phenylhydantoin (p-HPPH) enantiomers, a major metabolites of PHT, were measured (Lavy, 1998). It was found that the mean maximal elimination rate (Vmax) was 42% lower in the patients who were heterozygous for CYP2C9 (Leu359 allele), and the mean Michaelis-Menten constants (Km) in the heterozygous extensive metabolizers and the poor metabolizers of CYP2C19 were 22% and 54% higher than those without the mutations in CYP2C9/19 genes, respectively. R- and S- p-HPPH/PHT ratios were lower in patients with mutations in CYP2C9 or CYP2C19 gene than those in

patients without mutations. Although the hydroxylation capacity of PHT was impaired with mutations of CYP2C9/19, the impairment was greater for CYP2C9. In view of the clinical use of PHT, two important conclusions were derived from this population study. First, the serum PHT concentration in patients with the Leu359 allele in CYP2C9 would increase dramatically even at lower daily doses. Second, the patients with CYP2C19 mutations should be treated carefully at higher daily dose of PHT (Levy, 1998).

5. Genetic polymorphism of CYP

Genetic polymorphism is defined as the inheritance of a trait controlled by a single genetic locus with two alleles, in which the least common allele has a frequency of about 1% or greater (Lennard, 1990). The intersubject variability in metabolic rate is largely determined by genetic factors (Meyer and May, 1994). As a result, the metabolic conversion and excretion rate of drugs vary between individuals, from extremely slow to ultrafast (Jan and Linda,1999). For many drugs, four major phenotypes can be distinguished; PMs, intermediate metabolizers (IMs), EMs and ultrarapid metabolizers (UMs) (Meyer, 1996). EMs is a characteristic of the normal population while PMs is associated with accumulation of specific drug substrates. PM is typically an autosomal recessive trait requiring mutation and/or deletion of both alleles for phenotypic expression. UMs results in increased drug metabolism and is an autosomal dominant trait arising from gene amplification (Jan and Linda, 1999). Some CYP enzymes, including CYP2D6 and CYP2C19, are genetically polymorphic. Genetic polymorphism of CYP2D6 has been linked to three classes of phenotypes based on the extent of drug metabolism EMs, PMs and UMs. (Linder *et al.*, 1997). In contrast to the CYP2D6 polymorphism, no UMs has been demonstrated for CYP2C19 (Jan and Linda, 1999). CYP2C9 enzyme is also known to be polymorphic. Several mutant alleles have been characterized, probably associated with decreased enzyme activity (Kimura *et al.*, 1998). For other CYP enzymes involved in the metabolism and the elimination of drugs such as CYP2C19, CYP2C9 and CYP2D6 there is some evidence of genetic heterogeneity in the population (May, 1994).

The advantage of combining genotyping over phenotyping with therapeutic drug monitoring is that genotyping can predict the PM or UM drug metabolism phenotypes. This information can be used for dosage adjustment or selection of an alternative drug, which is not a substrate of that CYP particular enzyme.

5.1 Genetic polymorphism of CYP2C19

Genetic polymorphism of CYP2C19 in the past was well-known in the metabolism of the anticonvulsant mephenytoin which was discovered in the 1979 at Vanderbilt University. It was the first discovery of the CYP2Cs subfamily. It associated with the 4'-hydroxylation of *S*-mephenytoin (Küpfer and Branch, 1985). The availability of phenotyping and genotyping methods should help identify the adverse reaction and toxicity of drugs that metabolized by CYP2C19 and determine the doses of these drugs according to individual CYP2C19 genotype.

Individuals can be characterized as either EMs or PMs. Genetic polymorphism of CYP2C19 is one of major causes of individual and interethnic variations in drug response such as drug-induced toxicity or therapeutic failure. The discovery of CYP2C19 polymorphism created new interest in the role of pharmacogenetics in clinical pharmacology. It is the most extensively studies of genetic polymorphism because CYP2C19 is an important drug metabolizing enzyme in human that metabolizes most common clinically used drugs such as phenytoin, proton pump inhibitors such as omeprazole and lansoprazole, certain barbiturates such as hexobarbital and mephobarbital, antidepressants such as amitriptyline, antimalarial e.g. proguanil, propranolol and diazepam. The therapeutic effects of these drugs are assumed to depend on CYP2C19 genotype status. Many studies have shown that the genotypic differences in CYP2C19 affects on the cure rates of omeprazole and other proton pump inhibitors. The eradication rates in patients infected with *H. pylori* receiving triple therapy with a proton pump inhibitor, amoxicillin and clarithromycin were lower in the homozygous extensive compared with the heterozygous and PMs groups (Furuta *et al.*, 2001). The effect of omeprazole on intragastric pH significantly depends on *CYP2C19* genotype status (Furuta *et al.*, 1999). The genotype test of CYP2C19 in patients may be

useful for an optimal prescription of omeprazole and possibly other drugs (Furuta *et al.*, 1999).

Genetic polymorphism of CYP2C19 may be segregated into two distinct groups. The first one comprises inactivating polymorphisms that result in the premature termination of protein synthesis such as *CYP2C19*2 CYP2C19*3*. The other one consists of point mutations that result in single amino acid substitutions that prevent protein translation or substantially reduce their biological activity such as *CYP2C19*4, CYP2C19*5, CYP2C19*6, CYP2C19*7 and CYP2C19*8* (Gordon *et al.*, 1999).

5.2 Allele variants of CYP2C19

To date more than 16 allele variants of cytochrome P450 2C19 have been reported. *CYP2C19*2* and *CYP2C19*3* are the most two defective alleles which have been described as PMs phenotype in about 99% in Asians populations and about 87% in Caucasians (de morais *et al.*, 1994a,b).

5.2.1 *CYP2C19*1A* and *CYP2C19*1B*

*CYP2C19*1A* and *CYP2C19*1B* are the wild-type allele variants that produce normal enzymes (Romkes, 1991). The *CYP2C19*1B* is different from *CYP2C19*1A* by an adenine at nucleotide 991 is changed to guanine (Romkes *et al.*, 1991 and Richardson *et al.*, 1995) and it has mutation of nucleic acid in exon 1 which arises from substitution of cytosine to thymine at position 99 (Richardson *et al.*, 1995). This mutation is non-functional polymorphism that has no effect on enzyme production.

5.2.2 *CYP2C19*2*

*CYP2C19*2* is the principal genetic defect found in PMs of *S*-mephenytoin polymorphism (de Morais *et al.*, 1994). It is a single mutation in exon 5 of CYP2C19 gene arises from a guanine to adenine transposition at position 681, creating an aberrant splice site (de Morais *et al.*, 1994). This change alters the reading frame of the mRNA starting with amino acids 215 and produces a premature stop codon 20 amino acid downsteam (de Morais *et al.*, 1994). The enzyme have 234 of amino acid which does not combine with heme and resulted in a truncated, non-functional protein. This

defect accounts for about 75-83% of PM alleles in both Japanese and Caucasians subjects (de Morais *et al.*, 1994), 18% in Nigerians (Iyun *et al.*, 1990), 4% in black Shona Zimbabweans (Coolen *et al.*, 1995).

5.2.3 *CYP2C19*3*

*CYP2C19*3* mutation is a single-base pair transposition of a guanine to adenine at position 636 in exon 4 of CYP2C19, which creates a premature stop codon and produces a truncated 211-amino acids and inactive protein that lack the heme-binding regions as well as the majority of the putative substrate-binding regions (de Morais *et al.*, 1994b). This defective allele was found primarily in Asians (about 20-25% of PM alleles), but rare in Caucasians (about 1% of PM alleles) (de Morais *et al.*, 1994b; Brosen *et al.*, 1995; Ferguson *et al.*, 1998).

5.2.4 *CYP2C19*4*

*CYP2C19*4* mutation contributes to the PM phenotype in Caucasians (Ferguson *et al.*, 1998). This allele contains an adenine to guanine substitution at the first base of exon 1, changing in the initiation codon from ATG to GTG then leads to inhibition of protein translation (Ferguson *et al.*, 1998). To verify that *CYP2C19*4* represented a defective CYP2C19 allele, the initiation codon of normal *CYP2C19*1* cDNA was mutated to a GTG, and both cDNAs were expressed in yeast (de Morais *et al.*, 1994a,b). Recombinant CYP2C19 protein was detected by Western blot analysis of colonies transformed with *CYP2C19*1* cDNA, but not in those transformed with *CYP2C19*4* cDNA. The two cDNAs were also used in an *in vitro* coupled transcription/translation assay. CYP2C19 protein was translated only from the *CYP2C19*1* allele (Ferguson *et al.*, 1998). *CYP2C19*4* allele found in Caucasians is about 0.6% (Ronald *et al.*, 1998).

5.2.5 *CYP2C19*5*

*CYP2C19*5* is a rare mutation resulting Arg_{433} to Trp substitution in the heme-binding region (Gordon *et al.*, 1998). There are two defective alleles of *CYP2C19*5* including *CYP2C19*5A*, $C_{1297}T$ (Arg_{433} Trp) and *CYP2C19*5B*, $C_{99}T$, $A_{991}G$, $Ile_{331}Val$, C_{1297} T ($Arg_{433}Trp$), (Gordon *et al.*, 1998). *CYP2C19*5A* has been reported in a single Chinese

PMs outlier belonging to the Bai ethnic group (Xiao *et al.*, 1997). *CYP2C19*5B* (C99>T; A991>G, Ile331Val; C1297T, Arg433Trp) found in one of 37 Caucasian PMs (Gordon *et al.*, 1998). The frequencies of the *CYP2C19*5* alleles are low in Chinese (approximately 0.25% in the Bai ethnic group) and Caucasians (< 0.9%) (Gordon *et al.*, 1998). The Arg433 to Trp mutation in the heme-binding region essentially abolishes activity of recombinant *CYP2C19*5A* toward *S*-mephenytoin and tolbutamide, which is consistent with the conclusion that *CYP2C19*5* represents PMs alleles (Ibeanu *et al.*, 1998).

5.2.6 *CYP2C19*6*

*CYP2C19*6* allele was found in PMs in Swiss population (Gordon *et al.*, 1998). This mutation consists of a single base pair mutation which arises from a guanine to adenine transposition at position 395 in exon 3 resulting in a change from Arg to Gln at amino acid position 132 coding change that reduces recombinant CYP2C19 enzyme activity by about 98% (Ibeanu *et al.*, 1998b). *CYP2C19*6* allele accounts for about 1.4% of the defective allele in 37 Caucasian PMs (Ibeanu *et al.*, 1998). This allele had negligible activity toward two CYP2C19 substrates, mephenytoin and tolbutamide in a cDNA expression system (Ibeanu *et al.*, 1998).

5.2.7 *CYP2C19*7*

*CYP2C19*7* is a mutation allele which was found in PMs in Caucasian population which contains a single thymidine to adenine nucleotide transversion at the 5′donor splice site of intron 5 and resulting in splicing defect (Ibeanu *et al.*, 1999). *CYP2C19*7* allele accounted for about 1.3% of the 40 defective alleles in 37 putative Caucasian PMs (Gordon *et al.*, 1999).

5.2.8 *CYP2C19*8*

*CYP2C19*8* was found in two of the four France PMs lung cancer group subjects whose genotypes did not agree with their phenotypes (Benhamou et al., 1997). Sequencing of the exons and intron-exon junctions revealed that the subject was heterozygous for a T358C base change in exon 3, which resulted in the substitution of a tryptophan at position 120 for an arginine residue (Ibeanu *et al.*, 1999). The *CYP2C19*8*

mutation allele showed 11-fold lower activity than the wild-type *CYP2C19*1B* protein enzyrne (Ibeanu *et al.*, 1999). *CYP2C19*8* also exhibited 7-fold lower activity for the universal CYP2C substrate tolbutamide when compared with wild-type *CYP2C19*1B*, confirming that *CYP2C19*8* is a defective allele of CYP2C19 (Ibeanu *et al.*, 1999).

5.2.9 *CYP2C19*9*

*CYP2C19*9* is a mutation allele comprises a G to A substitution at neucleotide 431 resulting in a change from Arg to His at amino acid 144 (Blaisdell *et al.*, 2002). When expressed in a bacterial cDNA expression system, *CYP2C19*9* exhibited a modest decrease in the V_{max} for 4-hydroxylation of *S*-mephenytoin, and no alteration in its affinity for reductase (Blaisdell *et al.*, 2002).

5.2.10 *CYP2C19*10*

*CYP2C19*10* is a mutation allele comprises a C to T substitution at neucleotide 680 resulting in a change from Pro to Leu at amino acid 227. *CYP2C19*10* exhibited a dramatically higher K_m and lower V_{max} for mephenytoin (Blaisdell *et al.*, 2002).

5.2.11 *CYP2C19*11*

*CYP2C19*11* is a mutation allele comprises a G to A substitution at neucleotide 449 resulting in a change from Pro to Leu at amino acid 150 (Blaisdell *et al.*, 2002).

5.2.12 *CYP2C19*12*

*CYP2C19*12* is a mutation allele comprises a A to G substitution at neucleotide 991 resulting stop Cys at amino acid 491. *CYP2C19*12* was unstable and expressed poorly in a bacterial cDNA expression system. Clinical studies will be required to confirm whether this allele is defective in vivo (Blaisdell *et al.*, 2002).

5.2.13 *CYP2C19*13*

*CYP2C19*12* is a mutation allele comprises a C to T substitution at neucleotide 1228 resulting in a change from Arg to Cys at amino acid 410 (Blaisdell *et al.*, 2002).

5.2.14 *CYP2C19*14*

*CYP2C19*14* is a mutation allele comprises a T to C substitution at neucleotide 50 resulting in a change from Pro to Leu at amino acid 17 (Blaisdell *et al.*, 2002).

5.2.15 *CYP2C19*15*

*CYP2C19*15* is a mutation allele comprises a A to C substitution at neucleotide 55 resulting in a change from Ile to Leu at amino acid 19 (Blaisdell *et al.*, 2002).

5.2.16 *CYP2C19*16*

*CYP2C19*16* is a mutation allele comprises a C to T substitution at neucleotide 1324 resulting in a change from Arg to Cys at amino acid 442 (Blaisdell *et al.*, 2002).

*CYP2C19*9*, *CYP2C19*10* and *CYP2C19*12* occurs in African-Americans or individuals of African descent and represents new potentially defective alleles of *CYP2C19* which predicts to alter risk of these populations to clinically important drugs (Blaisdell *et al.*, 2002). The allelic variants of CYP2C19 are summary in Table 5.

Table 5 Allelic variants of CYP2C19

CYP2C19 allele nomenclature						
Allele	Nucleotide changes	Trivial name	Effect	Enzyme activity		References
				In vivo	*In vitro*	
*CYP2C19*1A*	None	wt1		Normal	Normal	Romkes *et al*, 1991
*CYP2C19*1B*	99C>T; 991A>G	wt2	I331V	Normal		Richardson *et al*, 1997
*CYP2C19*1C*	991A>G		I331V	Normal		Blaisdell *et al*, 2002
*CYP2C19*2A*	99C>T; 681G>A; 990C>T; 991A>G	m1; m1A	splicing defect	None		de Morais *et al*, 1994a
*CYP2C19*3*	636G>A; 991A>G; 1251A>C	m2	stop codon	None		de Morais *et al*, 1994b
*CYP2C19*4*	1A>G; 99C>T, 991A>G	m3	GTG initiation codon	None		Ferguson *et al*, 1998
*CYP2C19*5A*	1297C>T	m4	R433W	None	None	Xiao *et al*, 1997 Ibeanu *et al*, 1998a
*CYP2C19*5B*	99C>T; 991A>G; 1297C>T		I331V; R433W	None		Ibeanu *et al*, 1998a
*CYP2C19*6*	99C>T; 395G>A; 991A>G	m5	R132Q; I331V	None	None	Ibeanu *et al*, 1998b
*CYP2C19*7*	IVS5+2T>A		splicing defect	None		Ibeanu *et al*, 1999
*CYP2C19*8*	358T>C		W120R	None	Decrease	Ibeanu *et al*, 1999
*CYP2C19*9*	99C>T; 431G>A; 991A>G		R144H; I331V		Decrease	Blaisdell *et al* 2002
*CYP2C19*10*	99C>T; 680C>T; 991A>G		P227L; I331V		Decrease	Blaisdell *et al* 2002
*CYP2C19*11*	99C>T; 449G>A; 991A>G		R150H; I331V			Blaisdell *et al* 2002
*CYP2C19*12*	991A>G; 99C>T; 1473A>C		I331V; X491C; 26 Extra aa		Unstable	Blaisdell *et al* 2002
*CYP2C19*13*	991A>G; 1228C>T		I331V; R410C			Blaisdell *et al* 2002
*CYP2C19*14*	50T>C; 99C>T; 991A>G		L17P; I331V			Blaisdell *et al* 2002
*CYP2C19*15*	55A>C; 991A>G		I19L; I331V			Blaisdell *et al* 2002
*CYP2C19*16*	1324C>T		R442C			Morita *et al.*, in press, Drug Met. & Pharm

Source : website http:/www.imm.ki.se/CYPalleles/cyp2c19.htm

5.3 Ethnic differences of CYP2C19 polymorphism

The genetic polymorphism of CYP2C19 shows a marked interethnic difference in the incidence of the poor metabolism phenotype. Table 6 shows list the frequencies of PMs of *S*-mephenytoin 4-hydroxylation polymorphism in various ethnics.

Table 6. Ethnic differences in the frequencies of poor metabolizers of CYP2C19 (Tassaneeyakul *et al.*, 2000).

Ethnic group	Frequency of PMs (%)	Ethnic group	Frequency of PMs (%)
Asians of:		**Caucasians of:**	
China	17	Canada	4
India	21	Canada (Inuit)	2
Indonesia	15	Denmark	3
Japan	18-23	France	6
Korea	13	Greenland (west)	3
The Philippines	23	(East)	9
Vietnam	22	The Netherlands	2
Mid-East Asians of:		Portugal	5
Israel	3	Russia	1-8
Saudi Arabians	2	Spain	3
Africans of:		Sweden	1
Black Americans	2	Switzerland	3
Zimbabwe	4	Turkey	3
Ethiopia	4	U.S.A.	1
Tanzania	5	**Miscellaneous:**	
		Vanuatu	68