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Inhibition and Induction of Cytochrome P450 and the Clinical Implications

Jiunn H. Lin and *Anthony Y.H. Lu*

Drug Metabolism, Merck Research Laboratories, West Point, Pennsylvania, USA

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Abstract

The cytochrome P450s (CYPs) constitute a superfamily of isoforms that play an important role in the oxidative metabolism of drugs. Each CYP isoform possesses a characteristic broad spectrum of catalytic activities of substrates. Whenever 2 or more drugs are administered concurrently, the possibility of drug interactions exists. The ability of a single CYP to metabolise multiple substrates is responsible for a large number of documented drug interactions associated with CYP inhibition. In addition, drug interactions can also occur as a result of the induction of several human CYPs following long term drug treatment.

The mechanisms of CYP inhibition can be divided into 3 categories: (a) reversible inhibition; (b) quasi-irreversible inhibition; and (c) irreversible inhibition. In mechanistic terms, reversible interactions arise as a result of competition at the CYP active site and probably involve only the first step of the CYP catalytic cycle. On the other hand, drugs that act during and subsequent to the oxygen transfer step are generally irreversible or quasi-irreversible inhibitors. Irreversible and quasi-irreversible inhibition require at least one cycle of the CYP catalytic process.

Because human liver samples and recombinant human CYPs are now readily

available, *in vitro* systems have been used as screening tools to predict the potential for *in vivo* drug interaction. Although it is easy to determine *in vitro* metabolic drug interactions, the proper interpretation and extrapolation of *in vitro* interaction data to *in vivo* situations require a good understanding of pharmacokinetic principles.

From the viewpoint of drug therapy, to avoid potential drug-drug interactions, it is desirable to develop a new drug candidate that is not a potent CYP inhibitor or inducer and the metabolism of which is not readily inhibited by other drugs. In reality, drug interaction by mutual inhibition between drugs is almost inevitable, because CYP-mediated metabolism represents a major route of elimination of many drugs, which can compete for the same CYP enzyme.

The clinical significance of a metabolic drug interaction depends on the magnitude of the change in the concentration of active species (parent drug and/or active metabolites) at the site of pharmacological action and the therapeutic index of the drug. The smaller the difference between toxic and effective concentration, the greater the likelihood that a drug interaction will have serious clinical consequences. Thus, careful evaluation of potential drug interactions of a new drug candidate during the early stage of drug development is essential.

The cytochrome P450s (CYPs) constitute a superfamily of isoforms that play an important role in the metabolism of drugs. One of the many intriguing aspects of CYPs is that not only can they catalyse numerous oxidative reactions (including carbon hydroxylation, heteroatom oxygenation, dealkylation and epoxidation), but they can also metabolise an amazingly large number of lipophilic xenobiotics.[1] This is accomplished by multiple forms of CYP which have overlapping substrate specificities.[2] Each CYP isoform possesses a characteristic broad spectrum of catalytic activities of substrates (table I).[3,4]

Multiple drug therapy is a common therapeutic practice, particularly in patients with several diseases or conditions. Whenever 2 or more drugs are administered over similar or overlapping time periods, the possibility of drug interactions exist. Just as drugs can compete for protein binding sites, they can also compete for enzyme catalytic sites. The ability of a single CYP to metabolise multiple substrates is responsible for the large number of documented drug interactions associated with CYP inhibition.[5-7] The inhibition of drug metabolism by competition for the same enzyme may result in undesirable elevations in plasma drug concentrations. Thus, the inhibition of CYP enzymes is of clinical importance for both therapeutic and toxicological reasons. In addition, drug interactions can also occur as a result of the induction of several human CYPs following prolonged drug treatment.

The purpose of this paper is to review the mechanisms of inhibition and induction of CYP enzymes and their clinical implications. In addition, the pharmacokinetic concepts for extrapolation of *in vitro* inhibition data to *in vivo* situations are briefly highlighted. This review is by no means intended to be comprehensive, rather it is meant to illustrate the important points of the current understanding of CYP inhibition and induction, and their consequences.

1. Human Hepatic Cytochrome P450s (CYPs)

The CYPs comprise a superfamily of haemoproteins which contain a single iron protoporphyrin IX prosthetic group. This superfamily is subdivided into families and subfamilies that are defined solely on the basis of amino acid sequence homology. To date, at least 14 CYP gene families have been identified in mammals.[8] The mammalian CYP families can be functionally subdivided into

2 major classes, those that involve the biosynthesis of steroids and bile acids and those that primarily metabolise 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 oxidising centre (the haem iron), but differ by their protein structures.

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

Approximately 70% of human liver CYP is accounted for by CYP1A2, CYP2A6, CYP2B6, CYP2C, CYP2D6, CYP2E1 and CYP3A enzymes. Among these, CYP3A (CYP3A4 and CYP3A5) and CYP2C (CYP2C8, CYP2C9, CYP2C18 and CYP2C19) are the most abundant subfamilies, accounting for 30% and 20% of the total CYP, respectively. Other isoforms are minor contributors to the total CYP: CYP1A2 at 13%, CYP2E1 at 7%, CYP2A6 at 4%, CYP2D6 at 2% and CYP2B6 at 0.2%.[3,9,10]

In humans, genomic analyses suggest that at least 7 genes exist in the CYP2C subfamily.[11] CYP2C8 and CYP2C9 are the major forms, accounting for 35 and 60%, respectively, of total human CYP2C forms, while CYP2C18 (4%) and CYP2C19 (1%) are the minor forms of the human CYP2C subfamily.[12]

On the other hand, CYP3A4 and CYP3A5 have been identified in adult human liver microsomes. CYP3A4 is the most abundant CYP isoform, comprising approximately 25% of the total CYP, and plays a very important role in human metabolism. CYP3A5 is believed to be polymorphically expressed, appearing in about a quarter of the human

Table I. Major human liver cytochrome P450 (CYP) enzymes (data from Guengerich[3] and Parkinson[4])

CYP	Drug substrate	Marker substrate/ reaction	Inhibitor	Inducer
1A2	Paracetamol (acetaminophen), caffeine, ondansetron, phenacetin, tacrine, tamoxifen, theophylline	Phenacetin *O*-de-ethylation	Furafylline	Smoking, charred food
2A6	Coumarin, nicotine	Coumarin 7-hydroxylation	Ditiocarb sodium (diethyldithio-carbamate)	
2C9	Diclofenac, flurbiprofen, losartan, phenytoin, piroxicam, tienilic acid, tolbutamide, torasemide, (*S*)-warfarin	Tolbutamide methyl hydroxylation	Sulfaphenazole	Barbiturates, rifampicin (rifampin)
2C19	Diazepam, (*S*)-mephenytoin, omeprazole, pentamidine, propranolol, (*R*)-warfarin	(*S*)-mephenytoin 4′-hydroxylation		
2D6	Bufuralol, codeine, debrisoquine, desipramine, dextromethorphan, encainide, fluoxetine, haloperidol, imipramine, nortriptyline, paroxetine, propafenone, propranolol, sparteine	Bufuralol 1′-hydroxylation	Quinidine, ajmaline	
2E1	Paracetamol, caffeine, chlorzoxazone, enflurane, theophylline	Chlorzoxazone 6-hydroxylation	Ditiocarb sodium	Alcohol (ethanol), isoniazid
3A4	Benzphetamine, clarithromycin, codeine, cyclosporin, dapsone, diazepam, erythromycin, felodipine, tacrolimus, indinavir, lovastatin, midazolam, nifedipine, carbamazepine, losartan, quinidine, taxol, terfenadine, verapamil	Testosterone 6β-hydroxylation	Gestodene, troleandomycin, L-754,394, ketoconazole, itraconazole	Barbiturates, rifampicin, dexamethasone, carbamazepine

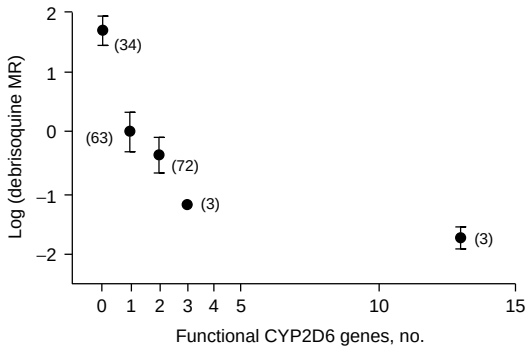

Fig. 1. Relationship between number of active cytochrome P450 (CYP) 2D6 genes and the metabolic ratio (MR) for debrisoquine. Number of individuals is indicated within parentheses.[18]

population. When CYP3A5 is expressed, the level is usually about one-third to one-quarter that of CYP3A4.[3] CYP3A7 is an enzyme that is expressed only in human fetal, but not adult, liver.[3]

There is considerable interindividual variation in the content of each CYP isoform. In a study with human liver microsomes of 30 Japanese and 30 Caucasians, Shimada et al.[9] have found that the interindividual difference in the content was 6-fold for CYP3A4, and 10- to 50-fold for CYP2A6 and CYP2D6, respectively. The mean values of CYP content were 42, 14, 60, 5, 22 and 96 pmol/mg microsomal protein for CYP1A2, CYP2A6, CYP2C8/9, CYP2D6, CYP2E1 and CYP3A4/5, respectively.

Interindividual variability in drug metabolism is a critical issue in drug therapy. Many factors contribute to the interindividual variability in drug metabolism. Among these, the genetic factor represents an important source of variability. Mutations in the gene for a drug metabolising enzyme could result in enzyme variants with higher, lower or no activity, or result in the absence of the enzyme. In recent years, significant progress has been made in understanding the role of genetic polymorphisms in drug metabolism. The major polymorphisms that have clinical implications are those related to the oxidation of drugs by CYP2D6 and CYP2C19.[13-15]

CYP2D6 polymorphism is perhaps the most studied genetic polymorphism in drug metabolism. This polymorphism divides the populations into 2 phenotypes, extensive metabolisers (EM) and poor metabolisers (PM). Approximately 5 to 10% of individuals in Caucasian populations are of the PM phenotype, but only 1 to 2% in Asian populations.[16,17] Recent studies by Johansson and colleagues[18,19] have shown that a small fraction of the Swedish population are ultrarapid metabolisers of debrisoquine, as the result of gene amplification or duplication. In 2 families of ultrarapid metabolisers, the CYP2D6 gene was amplified 12-fold on 1 allele in 3 members of 1 family, and 2 gene copies were present on 1 allele in another family. Examination of the metabolic ratio (MR) for debrisoquine revealed an excellent correlation between debrisoquine metabolism as measured by MR and the number of active genes (fig. 1). A high frequency (29%) of ultrarapid metabolisers of debrisoquine in an Ethiopian population carrying multiple CYP2D6 genes has also been reported.[20] To date, more than 50 drugs, including antidepressants, antipsychotics and cardiovascular drugs, are known to be catalysed primarily by CYP2D6.[4]

CYP2C19 also exhibits genetic polymorphism in drug metabolism. The incidence of the PM phenotype in populations of different racial origin varies; approximately 2 to 6% of individuals in the Caucasian population, and 18 to 22% in Asian populations.[21,22]

In general, a significant drug-drug interaction occurs only when 2 or more drugs compete for the same enzyme and when the metabolic reaction catalysed by this enzyme is a major elimination pathway. Drug-drug interactions can also occur when the CYP responsible for the metabolism of a drug is induced by long term treatment with another drug. Thus, definitive assessment of the role of an individual CYP in a given metabolic pathway is essential in determining and predicting the potential for drug interactions. To identify which CYP isoforms are responsible for the oxidative metabolism of drugs, a general strategy has emerged for *in vitro* studies.[23,24] This involves: (a) use of selec-

tive inhibitors; (b) immunoinhibition; (c) catalytic activity in cDNA-based vector systems; (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.

2. 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.[25,26]

Although impairment of any 1 of these steps can lead to inhibition of CYP enzyme activity, steps (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.[27,28] Among these, reversible inhibition is probably the most common mechanism responsible for the documented drug interactions. In mechanistic terms, reversible interactions arise as a result of competition at the CYP active site and probably involve only the first step of the CYP catalytic cycle. On the other hand, agents that act during or subsequent to the oxygen transfer step are generally irreversible or quasi-irreversible inhibitors. Both irreversible and quasi-irreversible inhibition are caused by the formation of reactive metabolites (sections 2.2 and 2.3). Thus, the irreversible and quasi-irreversible inhibition require at least 1 cycle of the CYP catalytic process.

2.1 Reversible Inhibition

Many of the potent reversible CYP inhibitors are nitrogen-containing drugs, 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.[29,30] 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.[31,32] The coordination of a strong ligand to the pentacoordinated iron, or the displacement of a weak ligand from the hexacoordinated haem by a strong ligand, gives rise to a 'type II' binding spectrum. However, cimetidine is a relatively weak reversible inhibitor of CYP, an apparent result of an intrinsic low binding affinity to microsomal CYP. This 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.[33]

Pyridine derivatives, like imidazoles, can interact with ferric CYP and elicit a type II binding spectrum.[34] The best known inhibitor among the pyridine derivatives is metyrapone. This compound acts as a potent and selective inhibitor of CYP isoforms, including the inhibition of 11β-hydroxylase that catalyses the final step in cortisol biosynthesis.[35] This inhibition of 11β-hydroxylase led to the use of metyrapone in the diagnosis and treatment of hypercortisolism (Cushing's syndrome) and other hormonal disorders.[36] Indinavir, an HIV protease inhibitor, contains a pyridine ring and is a potential inhibitor of CYP3A4. This drug competitively inhibits the oxidation

metabolism of clarithromycin, which is catalysed mainly by CYP3A4.[37]

The quinolines are another class of nitrogen heterocycles that exhibit potent CYP inhibition. Ellipticine is a quinoline-containing compound that interacts with both ferrous and ferric CYP forms.[38] Ellipticine and its derivative 9-hydroxy-ellipticine have been used successfully as selective inhibitors of CYP1A1/2 activity.[38]

Other quinoline derivatives include quinidine and its diastereoisomer quinine, both of which are potent reversible inhibitors of debrisoquine 4-hydroxylation, a reaction catalysed by the CYP2D subfamily.[39] However, quinidine is a more potent inhibitor of this activity in human liver microsomes than in rat liver microsomes, while the reverse is true for quinine. The inhibition constant of the inhibitor (K_i) values of quinidine for debrisoquine 4-hydroxylation in humans and rats were 0.6 μmol/L and 50 μmol/L, respectively, whereas with quinine the values were 13 μmol/L and 1.7 μmol/L, respectively.[39] The reason for the species difference in the specificity of quinidine and quinine is not yet known, but it could be because of differences in the geometry of the active site of the respective CYP isoforms in humans and rats. Interestingly, quinidine is a potent inhibitor of CYP2D6 in humans, but is metabolised by CYP3A4, not CYP2D6. Thus, a potent inhibitor of a given CYP isoform need not be a substrate of the isoform.

Many antimalarial agents (such as primaquine, chloroquine, amodiaquine and mefloquine) contain a quinoline ring and are potent reversible CYP inhibitors.[40,41] However, the inhibition activity is not associated with the quinoline structure, since the pyridine nitrogen is sterically hindered. Instead, the amino group in substituents on the quinoline ring appears to be the primary determinant of the observed inhibition potency. The terminal amino group in the 8-substituent of primaquine is believed to be involved in the direct binding to the haem iron of the ferric CYP.[40,42]

2.2 Quasi-Irreversible Inhibition via Metabolic Intermediate Complexation

A large number of drugs, including methylenedioxybenzenes, alkylamines, macrolide antibiotics and hydrazines, undergo metabolic activation by CYP enzymes to form inhibitory metabolites. These metabolites can form stable complexes with the prosthetic haem of CYP, called metabolic intermediate (MI) complex, so that the CYP is sequestered in a functionally inactive state. MI complexation can be reversed, and the catalytic function of ferric CYP can be restored by *in vitro* incubation with highly lipophilic compounds that displace the metabolic intermediate from the active site.[43,44] Other *in vitro* methods by which the ferrous complex can be disrupted include irradiation at 400 to 500nm or oxidation to the ferric state by the addition of potassium ferricyanide.[45] 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.

Piperonyl butoxide, a methylenedioxybenzene derivative, has been used for many years as an inhibitor of oxidative drug metabolism. This compound acts by forming an MI complex,[46] presumably a carbene-iron complex (fig. 2). The ferrous

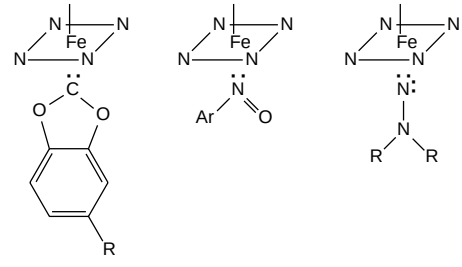

Fig. 2. Structures proposed for the metabolic intermediate complex formed during the catalytic turnover of (**left**) methylenedioxyphenyl compounds to carbene-iron complex, (**middle**) alkylamines to nitroso-iron complex and (**right**) 1,1-dialkylhydrazines to nitrene-iron complex.[24]

complex formed by methylenedioxybenzene derivatives is characterised by a distinct absorption spectrum with double maxima at 427 and 455nm, whereas the ferric complex has a single absorption maximum at 437nm.[47,48]

The nature of benzodioxole substitution is an important determinant of the inhibitory activity of methylenedioxybenzene derivatives. An electron withdrawing group causes a decrease in MI complex formation,[49] whereas an electron donating or a long-chain alkyl group favours MI complex formation.[46] Furthermore, depending on the dosage regimen, methylenedioxybenzene derivatives can act under certain conditions as inhibitors or inducing agents. Isosafrole, a methylenedioxybenzene derivative, which causes CYP inhibition after a single dose, induces CYP2E1 when administered to rats for 3 days.[50]

Troleandomycin and erythromycin are probably the best known macrolide antibiotics used as selective inhibitors which involve formation of the MI complex. These 2 agents are amino sugars bearing a tertiary amine function. Formation of the MI complex from a tertiary amine is mediated by CYP in several steps.[51] The sequence of *N*-demethylation, *N*-hydroxylation and *N*-oxidation produces a nitroso metabolite that binds tightly to the ferrous CYP (fig. 2) and gives rise to a spectrum with an absorbance maximum in the region of 445 to 455nm. Unlike the MI complexes of methylenedioxybenzene derivatives, the amines form MI complexes only with the ferrous CYP, a fact accounted for by the instability of their complexes in the ferric state. However, not all macrolide antibiotics form MI complexes. Steric hindrance around the tertiary amine group and the lipophilicity of the molecules are important factors in determining their potency as MI complex precursors. For instance, a second sugar attached to the amino sugar, by steric hindrance, reduces the formation of MI complex.[52]

Like methylenedioxybenzene derivatives, both troleandomycin and erythromycin act not only as inhibitors, but also as inducers. Repeated doses of troleandomycin induce CYP in male rats.[51] Most of the induced CYP isoenzymes are eventually complexed and inactivated *in vivo*. The concentration of remaining uncomplexed CYP depends both on the daily dose of troleandomycin[51] and the duration of administration.[53] Human CYP3A4 is among the isozymes induced by troleandomycin. In 6 humans treated with troleandomycin (2g daily for 7 days), NADPH-CYP reductase activity was increased by 48% and total CYP by 76% compared with that in a control group.[54] Again, most of the induced CYP was present in the form of MI complex. Similarly, administration of erythromycin led to a dose- and time-dependent increase in the NADPH-CYP reductase activity and total CYP in rats and humans.[55,56] The inducing effects of troleandomycin and erythromycin are caused by slow degradation of MI complexes. In rat liver and cultured rat hepatocytes, troleandomycin does not increase the rate of CYP3A protein synthesis; instead, it decreases the rate of CYP3A protein degradation to about a quarter of normal levels.[53] Because most of the induced CYP enzymes are complexed and not available for drug metabolism *in vivo*, the induction by MI complexation may be masked by inhibitory effects.

Another alkylamine drug associated with CYP complexation is orphenadrine, a muscle relaxant agent used in the treatment of Parkinson's disease. *In vivo* and *in vitro* studies have shown the induction and MI complexation of CYP2B1 in rats.[57] Because this drug contains a tertiary amine moiety, it is believed that metabolism of the alkylamine to a nitroso metabolite is the biotransformation reaction that generates the MI complex with CYP.

Proadifen (SKF-525A) was one of the first alkylamines shown to elicit MI complexation with CYP.[58] Although this compound has been widely regarded for many years as a universal inhibitor of all CYP, recent findings suggest that proadifen is not uniformly potent against the activity of all CYPs.[59] Proadifen generates complexes with rat CYP2B1, CYP2C11 and CYP3A1/2, but not with CYP2A1. Like other alkylamine compounds, proadifen also is an enzyme inducer when used long term.[58]

Hydrazine derivatives are another class of compounds that may elicit MI complexation with CYP. The nature of hydrazine substitution is an important determinant of complex formation. 1,1-Disubstituted hydrazines, in contrast to monosubstituted hydrazines, are oxidised by CYP to nitrene intermediates that bind tightly to the prosthetic haem iron to form the nitrene-iron complex[60] (fig. 2). Isoniazid, the hydrazide of isonicotinic acid, also elicits MI complexation.[61] CYP complexation may explain in part why isoniazid, which is metabolised predominantly by *N*-acetylation in humans, inhibits the CYP-mediated metabolism of phenytoin and warfarin.[62,63]

2.3 Irreversible Inactivation of CYP

Drugs containing certain functional groups can be oxidised 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.[64] 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.[65]

2.3.1 Haem Alkylation

Drugs containing terminal double-bond (olefins) or triple-bond (acetylenes) can be oxidised by CYP to radical intermediates that alkylate the prosthetic haem group and inactivate the enzyme.[27,65] The evidence for haem alkylation includes the demonstration of equimolar loss of enzyme and haem, as well as the isolation and structural characterisation of the haem adducts. Haem alkylation is initiated by the addition of activated oxygen to the internal carbon of the double or triple bond and is terminated by binding to haem pyrrole nitrogen. 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.[27]

Allylisopropylacetamide (AIA), an olefinic derivative, is a classic suicide substrate of CYP. This compound is now recognised as an effective haem-alkylating inactivator of rat CYP2B1 and CYP3A1, with CYP2C6 and CYP2C11 being less susceptible.[66] Interestingly, AIA-inactivated CYP isoforms can be restored partially by *in vitro* and *in vivo* haem supplementation. Administration of exogenous haem *in vivo* to phenobarbital-treated rats given AIA or addition of haem *in vitro* to liver homogenates from such rats resulted in partial restoration of CYP2B1 and CYP3A1 activity and, to a lesser extent, of CYP2C6 and CYP2C11.[67]

Ethinylestradiol, an acetylenic derivative, is an orally active estrogen widely used in oral contraceptives. Unlike other estradiol derivatives, ethinylestradiol has long biological activity and good bioavailability. Studies by Guengerich[68] have indicated that this drug is a substrate for human CYP3A4 and also elicits mechanism-based destruction of the enzyme. It is now clear that the good activity and bioavailability of ethinylestradiol is mainly attributed to its destruction of the prosthetic haem of the enzyme that metabolises it.

Like olefins and acetylenes, dihydropyridines also can be oxidised by CYP to reactive metabolites that alkylate the prosthetic haem. For example, 4-alkyl-1,4-dihydropyridines are oxidised by CYP enzymes to radical cation intermediates that *N*-alkylate the prosthetic haem group of CYP.[69] Not all dihydropyridines elicit haem alkylation; the substitution at position 4 of the dihydropyridine ring is an important determinant. Haem alkylation is detected if the substitution at position 4 is a primary, unconjugated moiety (methyl, ethyl, propyl), but not if it is an aryl (phenyl), secondary (isopropyl) or conjugated (benzyl) group.[27,69] For example, nifedipine, the 4-aryl-substituted dihydropyridine, does not inactivate CYP at all.

2.3.2 Covalent Binding to Apoprotein

The best known example of inactivation of CYP through protein modification by a suicide inactiv-

ator is that of chloramphenicol. The dichloroacetamido group is oxidised to an oxamyl moiety that acylates a lysine residue in the CYP active centre.[70] This acylation event interferes with the transfer of electrons from CYP reductase to the haem group of the CYP and thereby prevents catalytic turnover of the enzyme.[71] The inactivation by chloramphenicol is not uniform for all CYPs. Studies with rat liver microsomes revealed that CYP2B1, CYP2C6 and CYP2C11 are susceptible to inactivation by chloramphenicol, whereas CYP1A1 and CYP1A2 are resistant.[72]

Although terminal acetylenes have been known to alkylate the prosthetic haem group, some terminal acetylene compounds, such as 2-ethynylnaphthalene, inactivate CYP by binding covalently to the protein with little loss of the haem group. 2-Ethynylnaphthalene is converted by CYP2B1 to a ketene, which modifies an active site peptide that includes Thr-302, a highly conserved residue known to play a role in oxygen activation.[73]

Oxidation of sulphur groups in drug molecules can result in the modification of the CYP protein. A variety of sulphur compounds inactivate CYP by binding covalently to protein after they are oxidatively activated by the enzyme. CYP inactivation by sulphur compounds is believed to be involved with sulphur oxidation that generates reactive sulphur metabolites. Tienilic acid, a substituted thiophene, is oxidised by yeast-expressed human CYP2C9 to a reactive metabolite, presumably a thiophene sulphoxide that binds covalently to the CYP apoprotein.[74]

The protein modification is caused by formation of a sulphur reactive metabolite, rather than formation of hydrodisulphides (RSSH). Although 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.[74] In addition, diallyl sulphide, a flavour component of garlic, is known to be a potent suicide inhibitor of CYP2E1.[75] The mechanism by which diallyl sulphide inhibits CYP2E1 involves initial oxidation at sulphur to give diallyl sulphone, which then undergoes metabolic activation on 1 or other of the terminal olefin groups to produce the ultimate reactive species.

Similarly, oxidation of nitrogen groups on drug molecules can result in modification of the CYP protein. For example, the cyclopropylamines are metabolised to reactive metabolites, which inactivate CYP.[76] Although the exact mechanism of the inactivation is still unclear, radiolabelling experiments show that the cyclopropylamines are covalently bound to the protein. It is proposed that these compounds are oxidised by CYP to form an aminium ion that undergoes ring expansion to the substituted azetidine that binds irreversibly to the apoprotein of the enzyme.[76]

The data on chloramphenicol, tienilic acid and cyclopropylamines clearly show that CYP enzymes can generate reactive species that modify the protein. In addition, some drugs can simultaneously modify both the apoprotein and the prosthetic haem group of CYP enzymes. For example, spironolactone, a thiosteroid used as a diuretic and antihypertensive agent, is known to be a suicide inactivator of the CYP2C and CYP3A subfamilies.[77] The inactivation of CYP by spironolactone occurs after hydrolysis of the 7α-thioester to give the free thiol. CYP then oxidises the thiol group to an electrophilic thiosteroid species that binds covalently to the protein and modifies the prosthetic haem group.[27]

3. Mechanisms 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,[78,79] only

in recent years have we 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,[80,81] some nontranscriptional mechanisms also are known to be involved. For example, troleandomycin induces human CYP3A4, but the mechanism is not transcriptional.[53] Troleandomycin produces no increase in the rate of CYP3A4 protein synthesis, but it decreases the rate of CYP3A4 protein degradation. Similarly, induction of CYP2E1 by alcohol (ethanol), acetone and isoniazid is caused by a nontranscriptional mechanism.[82,83] Spontaneously induced diabetic rats and rats with chemically induced diabetes exhibit increased levels of CYP2E1 that appear to reflect mRNA stabilisation and not gene transcription.[84,85]

For many years, scientists have been trying to solve the mystery of how the cells recognise the inducing agents and how the signal is transferred to the transcriptional machinery. With the exception of the CYP1A1 isoform, the molecular mechanisms involved in CYP induction are still not fully understood. In the case of CYP1A1, inducing agents bind to cytosolic polycyclic aromatic hydrocarbon (Ah) receptors and are translocated into the nucleus. The transcriptional process includes a sequence of events: ligand-dependent heterodimerisation between the Ah receptor and an Ah receptor nuclear translocator, interaction of the heterodimer with a xenobiotic-responsive enhancer, transmission of the induction signal from the enhancer to a CYP1A1 promoter, and alteration in chromatin structure. This is followed by subsequent transcription of the appropriate mRNA and translation of the corresponding proteins.[80,81]

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 metabolising enzymes may lead to a decrease in toxicity through acceleration of detoxification, or to an increase in toxicity caused by increased formation of reactive metabolites. Depending upon the delicate balance between detoxification and activation, induction can be a beneficial or harmful response.

In vivo, the induction of CYP1A isoforms can reduce the carcinogenicity of certain compounds. For example, intraperitoneal injection of the CYP1A inducer β-naphthoflavone inhibited tumorigenesis in the lung and mammary glands of rodents treated with 7,12-dimethylbenz[a]anthracene (DMBA), which is a highly carcinogenic compound.[86] In addition, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a potent CYP1A inducer, dramatically reduced the initiation of skin tumours in mice caused by DMBA.[87] In contrast, CYP1A isoforms can activate some compounds, such as benzo[a]pyrene, to their ultimate carcinogenic species,[88] and induction of these isoforms increases the risk of carcinogenicity. Because of the complexity of the factors determining toxicity and carcinogenicity, the issue of whether induction is beneficial or harmful is still highly controversial.[89,90]

In addition to the induction of CYP1A isoforms, the binding of inducing agents to the Ah receptor sometimes also leads to the induction of UDP glucosyltransferases (UGTs) and glutathione (GSH)-*S*-transferases.[91] The co-induction of phase I and phase II enzymes appears to decrease the risk caused by CYP induction alone. *In vitro* mutagenicity of benzo[a]pyrene and benzo[a]pyrene-3,6-quinone was higher in the liver S9 fraction of rats treated with 3-methylcholanthrine (3-MC) than in control rats when NADPH was the only added cofactor. The *in vitro* mutagenicity was substantially decreased by concomitant glucuronidation or GSH conjugation when UDP glucuronic acid or GSH was added to the system; there was no significant difference in the *in vitro* mutagenicity between rats treated with 3-MC and the control group.[91] Thus, the protective effect appeared to be a result of co-induction of UGTs and GSH-*S*-transferases.

Although CYP1A in different species, including mice, rats, rabbits and humans, can be induced by various agents, there are important quantitative differences in the effectiveness of inducer-receptor coupling. For example, the gastric acid–suppressing drug, omeprazole, is a CYP1A2 enzyme inducer in humans, but has no such inductive effect in mice or rabbits.[92,93] Important interspecies differences also exist in the response of other inducible subfamilies of CYP. Phenobarbital induces predominantly members of the CYP2B subfamily in rats, whereas in humans it appears that the major form induced belongs to the CYP3A subfamily.[94] Furthermore, members of the CYP3A subfamily in rats are inducible by the steroidal agent, pregnenolone-16α-carbonitrile, but not by the antibiotic rifampicin (rifampin). The opposite is true in rabbits and humans.[95,96] Thus, drugs that induce CYP enzymes in animals should not be assumed necessarily to have enzyme-inducing capacity in humans, and vice versa.

4. Drug-Drug Interaction

A drug interaction occurs when the disposition of one drug is altered by another. Because oxidative metabolism represents a major route of elimination for many drugs, and because many drugs can compete for the same enzyme, inhibition of CYPs is one of the main reasons for drug interactions. Because of the potential of adverse effects, metabolic drug interaction has always been an important aspect to consider during the development of new drugs (see section 5 regarding dosage adjustment).

In the past, most drug interaction studies were conducted relatively late in the phase II and III clinical studies using a strategy based on the therapeutic indices of drugs and the likelihood of their concurrent use. Since drug-drug interaction is normally considered to be an undesirable property of drugs, the information on CYP inhibition ideally should be obtained earlier, before the selection of a drug candidate for development.

With the availability of human tissues and recombinant human CYP enzymes, *in vitro* systems have been used in recent years as screening tools to predict the potential *in vivo* drug interaction at a much earlier stage.[97,98] In fact, the use of *in vitro* systems for investigating the ability of a drug to inhibit the metabolism of other drugs provides some of the most useful information in predicting potential drug-drug interactions. Many pharmaceutical companies now use *in vitro* techniques to assess potential drug interactions as part of their screening processes in the selection of new drug candidates for development.

4.1 Enzyme Kinetic Considerations

As discussed in section 2, enzyme inhibition can be divided into reversible and irreversible processes (metabolic intermediate complexation and enzyme inactivation). Changes to drug disposition will be quite different depending on whether enzyme inhibition is a reversible or irreversible process. In order to distinguish between these 2 processes, and to design appropriate *in vitro* experimental conditions, an understanding of enzyme inhibition kinetics is necessary.

Kinetically, reversible inhibition can be classified further as a competitive, noncompetitive or uncompetitive process. For competitive inhibition, the binding of the inhibitor prevents binding of substrate to the active site of free enzyme, while for noncompetitive inhibition the inhibitor binds to another site of the enzyme and the inhibitor has no effect on binding of substrate, but the enzyme-substrate-inhibitor complex is nonproductive. In the case of uncompetitive inhibition, the inhibitor does not bind the free enzyme, but binds to the enzyme-substrate complex, and again the enzyme-substrate-inhibitor complex is nonproductive.[99]

For Michaelis-Menten kinetics, the velocity of an enzymatic reaction in the absence (v_o) of inhibitor can be described by equation 1 and in the presence (v_i) of inhibitor can be expressed by equations 2, 3 and 4 for competitive, noncompetitive and uncompetitive inhibition, respectively.[99]

$$v_o = \frac{V_{max} \cdot [S]}{Km + [S]} \quad \text{(Eq. 1)}$$

$$v_i = \frac{V_{max} \cdot [S]}{Km\left(1 + \frac{[I]}{K_i}\right) + [S]} \quad \text{(Eq. 2)}$$

$$v_i = \frac{V_{max} \cdot [S]}{Km\left(1 + \frac{[I]}{K_i}\right) + [S]\left(1 + \frac{[I]}{K_i}\right)} = \frac{\frac{V_{max}}{1 + \frac{[I]}{K_i}} \cdot [S]}{Km + [S]} \quad \text{(Eq. 3)}$$

$$v_i = \frac{V_{max} \cdot [S]}{Km + [S]\left(1 + \frac{[I]}{K_i}\right)} = \frac{\frac{V_{max}}{1 + \frac{[I]}{K_i}} \cdot [S]}{\frac{Km}{1 + \frac{[I]}{K_i}} + [S]} \quad \text{(Eq. 4)}$$

where V_{max} is the maximum velocity of metabolism, Km is the Michaelis-Menten constant of the substrate, K_i is the inhibition constant of the inhibitor, and [S] and [I] are the substrate and inhibitor concentrations, respectively. As indicated in equations 2 and 3, a competitive inhibitor acts only to increase the apparent Km and has no effect on the V_{max}, while a classic noncompetitive inhibitor decreases the V_{max}, but has no effect on the Km. On the other hand, an uncompetitive inhibitor decreases both the V_{max} and Km to the same extent (equation 4).

By the rearranging of equations 1 and 2, 3 or 4, the percentage of inhibition (PI) can be described as equations 5, 6 and 7 for competitive, noncompetitive and uncompetitive, respectively.

$$PI = \frac{v_o - v_i}{v_o}(\%) = \frac{\frac{[I]}{K_i}}{1 + \frac{[I]}{K_i} + \frac{[S]}{Km}} \quad \text{(Eq. 5)}$$

$$PI = \frac{v_o - v_i}{v_o}(\%) = \frac{\frac{[I]}{K_i}}{1 + \frac{[I]}{K_i}} \quad \text{(Eq. 6)}$$

$$PI = \frac{v_o - v_i}{v_o}(\%) = \frac{\frac{[I]}{K_i}}{1 + \frac{[I]}{K_i} + \frac{Km}{[S]}} \quad \text{(Eq. 7)}$$

As shown in equations 5 and 7, the degree of inhibition caused by a competitive or an uncompetitive inhibitor depends on, [S], [I], Km and K_i, while the degree of inhibition by a noncompetitive inhibitor depends only on [I] and K_i as indicated in equation 6.

From equations 5 to 7, it is clear that the inhibitor concentration that inhibits drug activity by 50% (IC_{50}) is not equivalent to the K_i values except in noncompetitive inhibition. Thus, an understanding of the class of inhibition and the relationship between the [I] and K_i, and the [S] and Km values is critical to the experimental design and interpretation of *in vitro* interaction studies.

Although mechanism-based inactivation and MI complexation are irreversible processes, they obey saturation kinetics. A characteristic time-dependent loss of enzyme activity is always observed. In fact, the time-dependent phenomenon is one of the most important criteria in distinguishing between reversible and irreversible inhibition. Preincubation of an irreversible inhibitor with the enzyme prior to the addition of substrate results in a time-dependent loss of enzyme activity towards the substrate, while a reversible inhibitor has no time-dependent effect on enzyme activity.

The general scheme for mechanism-based inactivation and MI complexation can be described as follows:

$$E + I \underset{K_{-1}}{\overset{K_1}{\leftrightarrow}} E \cdot I \xrightarrow{K_2} E \cdot I' \xrightarrow{K_4} E - II''$$
$$\downarrow K_3$$
$$E + P$$

(Eq. 8)

where E is the concentration of active enzyme and I is the concentration of inactivator. When an enzyme catalyses the inactivator to its reactive form I′, the reactive species either can be released as a product (P) or react with the enzyme to form E–II′, resulting in the inactivation of the enzyme. K_1, K_{-1}, K_2, K_3 and K_4 are individual reaction rate constants. As mentioned earlier, an important consequence related to mechanism-based inactivation is time-dependent loss of enzyme activity. The important kinetic parameter, half-life of enzyme inactivation ($t_{½}$), is described by equation 9:[64]

$$t_{½} = \frac{0.693}{K_{inact}}\left(1 + \frac{K_I}{I}\right) \quad \text{(Eq. 9)}$$

where K_{inact} is the rate constant of inactivation and K_I is the concentration of inactivator that produces half the maximal rate of inactivation. K_{inact} and K_I can be described in the following equations in terms of individual rate constants (eq. 8):

$$K_{inact} = \frac{K_2 K_4}{K_2 + K_3 + K_4} \quad \text{(Eq. 10)}$$

$$K_I = \left(\frac{K_{-1} + K_2}{K_1}\right)\left(\frac{K_3 + K_4}{K_2 + K_3 + K_4}\right) \quad \text{(Eq. 11)}$$

The reactivity of an inactivator can be reflected by $t_{½}$. The shorter the $t_{½}$ of an inactivator, the greater its potency as an enzyme inactivator. Experimentally, K_{inact} and K_I can be measured by using equation 9. The $t_{½}$ for enzyme inactivation is measured in a series of experiments in which the inactivator concentration [I] is varied. A plot of [I] × $t_{½}$ *vs* [I] is linear, and the K_{inact} and K_I can be obtained from the slope and intercept, respectively.[64]

In the case of mechanism-based inactivation and MI complexation, a fraction of CYP enzymes is destroyed or complexed, but the remaining enzymes should be intact with normal enzyme activity. Thus, a compound that elicits enzyme inactivation or MI complexation will result in a time-dependent decrease in V_{max}, but will have no effect on Km. The degree of inactivation by an irreversible inactivator depends not only on the concentration of inactivator, but also on the time of incubation. *In vivo*, the degree of inactivation depends on the dose and the duration of administration.

In contrast to enzyme inactivation and MI complexation, enzyme induction increases the enzyme levels in a time-dependent manner because induction is a slow regulatory process. This means that induction increases the V_{max} of metabolic reaction, but has no effect on the Km. Similarly, the degree of inducibility depends not only on the dose of inducers, but also on the duration of administration. Because the induction is a dose- and time-dependent phenomenon, Levy et al.[100-102] have derived equations to describe the time-course of induction under a variety of input conditions. They introduced a new pharmacokinetic parameter, the induction half-life, to describe the time-dependency of induction. This parameter provides a means of quantifying the interindividual variability in time dependency of enzyme induction.

4.2 *In Vitro* Drug Interaction

Drug metabolism is a complex process, which very often involves several pathways and various enzyme systems. In some cases, all the metabolic reactions of a drug are catalysed by a single enzyme, while in other cases a single metabolic reaction may involve multiple isoforms or different enzyme systems. The metabolism of indinavir, an HIV protease inhibitor, exemplifies the first scenario in which a single isoform of CYP, CYP3A4, catalyses 4 oxidative metabolic reactions, *N*-oxidation, *N*-dealkylation, indan hydroxylation and phenyl hydroxylation to produce 6 metabolites in human liver microsomes.[103] On the other hand, the

S-oxidation of 10-(*N*,*N*-dimethylaminoalkyl) phenothiazines in human liver microsomes is catalysed by numerous CYP isoforms, including CYP2A6, CYP2C8 and CYP2D6.[104] Therefore, definitive identification of the CYP isoforms responsible for drug metabolism is essential in predicting the potential drug interactions.

In assessing the consequences of drug interaction, 2 important factors must be considered:
(i) the identity of CYP isoforms responsible for metabolising the involved drugs
(ii) the relative contribution of the metabolic pathways being inhibited to the overall elimination of the drug.

Over the last 10 years, a great deal of information on human CYP at the molecular level has become available. This information, along with available antibodies and chemical inhibitors, has made it possible to easily determine the CYP isoforms responsible for the metabolism of a drug.[23,24] In addition to the identification of the CYP isoforms, it is also important to evaluate the relative contributions of the metabolic pathways being inhibited (or induced) to the overall elimination of the drug. With the advent of commercial liquid chromatography/mass spectrometry instrumentation and the development of high-field nuclear magnetic resonance as well as liquid chromatography/nuclear magnetic resonance techniques, the metabolite profile of a drug can be obtained readily in both qualitative and quantitative terms.

Although the identification of CYP isoforms is relatively straightforward, the interpretation of *in vitro* interaction studies can be complicated. One of the important factors in *in vitro* drug interaction studies is the use of clinically relevant concentrations of inhibitor and substrate. The use of supratherapeutic drug concentrations may produce drug interaction *in vitro*, but not *in vivo*. In addition, the major metabolic pathway may depend on the drug concentration used. For example, *N*-demethylation is the major metabolic pathway for diazepam in humans receiving clinical dosages. However, *in vitro* studies in human liver microsomes showed that 3-hydroxylation was the major pathway when a high drug concentration (100 μmol/L) was employed.[105] The *in vitro* and *in vivo* discrepancy are caused by the differences in the drug concentration used *in vitro* and observed *in vivo*. Indeed, *N*-demethylation is the major metabolic pathway of diazepam in human liver microsomes when a clinically relevant drug concentration (2 μmol/L) is used.[106] It should be noted that *N*-demethylation of diazepam is catalysed by CYP2C19 and 3-hydroxylation is mediated by CYP3A4. This example illustrates the importance of the use of drug concentration in *in vitro* drug interaction studies in order to define the involved CYP isoforms and to predict the *in vivo* situation.

Another important factor in in vitro drug interaction studies is the protein concentration of microsomes. The Ki values of an inhibitor may be overestimated when a high microsomal protein concentration is used as a result of the depletion of the inhibitor by nonspecific binding to microsomal proteins and microsomal metabolism. The K_i values for ketoconazole-CYP3A4 interactions in human liver microsomes were estimated to be about 8 μmol/L when a high microsomal protein concentration (1.5 g/L) was used,[107] while the estimated K_i values were about 0.03 μmol/L when a low microsomal protein concentration (0.25 g/L) was employed.[108,109] A 6-fold increase in the microsomal protein concentration resulted in a 270-fold increase in the estimated K_i values.

The use of *in vitro* enzyme systems, such as liver microsomes, cDNA-based vector systems and liver slices, are also important factors affecting *in vitro* drug interaction studies. For instance, the apparent Km characterising the hydroxylation of ritonavir, a potent HIV protease inhibitor, in β-lymphoblastoid-derived microsomes was similar to that obtained with human liver microsomes. However, the apparent Km characterising the *N*-dealkylation and decarbamoylation of ritonavir was 30- to 300-fold lower in β-lymphoblastoid-derived microsomes than in human liver microsomes.[110] The reason for this discrepancy is unknown, but it is clear that we should be cautious in interpreting the kinetic

parameters obtained from different *in vitro* systems.

Similarly, care should be taken to interpret kinetic results from liver slices. Worboys and associates[111] have shown that the values of intrinsic clearance (V_{max}/Km) of a series of drugs in liver slices are consistently lower than those in hepatocytes, by a factor ranging from 2 to 20. Similar results have been reported by other investigators. Human liver microsomes metabolised phenytoin,[112] cyclosporin[113] and an ergot derivative[114] several times faster than human liver slices, and clearance predictions from microsomes were closer to *in vivo* values than were predictions from slices. These results strongly suggest that a distribution equilibrium is not achieved between all the cells within the slice and the incubation media, probably caused by the slice thickness (≈260μm). In fact, Dogterom[115] has found that increased slice thickness resulted in a decrease in the rates of metabolism when normalised to slice wet weight.

Furthermore, an understanding of the mechanism involved in enzyme inhibition is crucial to providing a rational basis for designing experimental conditions and interpreting drug interaction data. For example, a compound that irreversibly inactivates an enzyme will result in a decrease in the V_{max}, but has no effect on the Km. The kinetic data are similar to that of a reversible noncompetitive inhibitor, which causes a decrease in the V_{max}, but not the Km. Thus, an irreversible inhibitor can be incorrectly referred to as a reversible noncompetitive inhibitor. The experimental results reported by Franklin[47] are a good example. Depending on the experimental conditions, proadifen acts as a competitive inhibitor or MI complexation-inducing agent. As shown in table II, proadifen increased the Km values of substrates, but had little effect on the V_{max} values when incubated with substrates without preincubation of the inhibitor. In contrast, proadifen decreased the V_{max} values of substrates and had little effect on the Km values when proadifen was preincubated prior to substrate addition. Thus, preincubation of proadifen changed the kinetics of inhibition from the reversible competitive type to irreversible MI complexation.

As mentioned in section 3, assessment of enzyme induction in animals may be of little clinical relevance in humans, because of the well-known interspecies differences in response to inducers.[92-96] Thus, *in vitro* methods provide an alternative approach to predicting enzyme induction in humans. Recently, *in vitro* techniques have been developed to evaluate enzyme induction by using cultured human hepatocytes.[116,117] The major disadvantage of using hepatocytes to assess enzyme induction is the potential for artifacts. For example, cultured rat hepatocytes are not inducible by streptozotocin, which induces CYP2E1 *in vivo*.[20] Furthermore, the CYP induction in cultured hepatocytes is highly dependent on the *in vitro* experimental conditions. Phenobarbital-inducibility of rat CYP2B1, CYP2B2, and CYP3A1 genes is maintained only in a specific medium containing extracellular matrix.[118]

Table II. Inhibition of rat hepatic microsomal monooxygenase activity by proadifen (SKF-525A) with or without preincubation prior to substrate addition[47]

Reaction	Inhibitor	No preincubation		Preincubation (5 minutes)	
		Km (mmol/L)	V_{max} (nmol/min/mg protein)	Km (mmol/L)	V_{max} (nmol/min/mg protein)
Aminophenazone (aminopyrine) *N*-demethylation	None	1.25	4.0	1.9	2.8
	Proadifen 50 μmol/L	7.10	4.0	1.9	1.4
Aniline *p*-hydroxylation	None	0.09	0.67	0.16	0.73
	Proadifen 50 μmol/L	0.25	0.67	0.16	0.33
Ethylmorphine *N*-demethylation	None	0.30	20	0.26	18
	Proadifen 17 μmol/L	0.72	17	0.31	4

Km = Michaelis-Menten constant; **V_{max}** = maximum velocity of metabolism by an enzyme-mediated reaction

4.3 *In Vitro*/*In Vivo* Extrapolation

Although it is relatively easy to assess *in vitro* drug interaction, the correct prediction and extrapolation of *in vitro* interaction data to the *in vivo* situations requires a good understanding of pharmacokinetic principles. In this section, we discuss some basic tenets of the effects of enzyme inhibition and induction on pharmacokinetics.

If a drug is mainly metabolised by the liver, the total clearance is approximately equal to the hepatic clearance (CL_H) which can be expressed as equation 12:[119]

$$CL_H = Q_H \cdot E = Q_H \cdot \left(\frac{f_u \cdot CL_{int}}{Q_H + f_u \cdot CL_{int}} \right) \quad \text{(Eq. 12)}$$

where Q_H is the hepatic blood flow, E is the hepatic extraction ratio, f_u is the unbound fraction of drug in blood and CL_{int}, the intrinsic clearance, is a measure of the drug metabolising activity (V_{max}/Km) in the liver. Depending on the underlying mechanism of the inhibitor, the V_{max} value of a drug can be decreased or the Km value can be increased. Thus, regardless of the mechanism, enzyme inhibition always results in a decrease in the intrinsic clearance (V_{max}/Km). On the other hand, enzyme induction always causes an increase in intrinsic clearance, as a result of increased V_{max}. Therefore, the concept of intrinsic clearance is the cornerstone for the extrapolation of *in vitro* data to the *in vivo* situation.

Drugs can be classified by whether their hepatic clearance is enzyme-limited (low) or flow-limited (high).[120] When the intrinsic clearance of a drug is very small relative to the hepatic blood flow ($Q_H >> f_u \cdot CL_{int}$), the hepatic clearance is low and is directly related to f_u and CL_{int} as shown in equation 13:

$$CL_H = f_u \cdot CL_{int} \quad \text{(Eq. 13)}$$

Thus, a change (decrease or increase) in the CL_{int} caused by inhibition or induction will result in an almost proportional change in the clearance of 'low clearance' drugs. On the other hand, if the intrinsic clearance is so high that $f_u \cdot CL_{int} >> Q_H$, then the hepatic clearance is limited by the hepatic blood flow as shown in equation 14:

$$CL_H = Q_H \quad \text{(Eq. 14)}$$

Thus, a change (decrease or increase) in the intrinsic clearance caused by inhibition or induction has little effect on the hepatic clearance of 'high clearance' drugs.

Because the hepatic first-pass metabolism reflects the hepatic intrinsic clearance (CL_{int}), systemic bioavailability (F) can be expressed as (equation 15):

$$F = 1 - E = \frac{Q_H}{Q_H + f_u \cdot CL_{int}} \quad \text{(Eq. 15)}$$

As shown in equation 15, the pharmacokinetic consequences of enzyme inhibition would be a decrease in first-pass metabolism resulting in an increased bioavailability, while enzyme induction would decrease the bioavailability. The simplest way of considering the effect of enzyme inhibition or induction on the plasma concentration of drugs is to examine the area under the concentration-time curve (AUC). The AUC after oral (PO) and intravenous (IV) administration can be expressed as (equations 16 and 17):

$$AUC_{PO} = \frac{F \cdot f_a \cdot Dose}{CL_H} = \frac{f_a \cdot Dose}{f_u \cdot CL_{int}} \quad \text{(Eq. 16)}$$

$$AUC_{IV} = \frac{Dose}{CL_H} = \frac{Dose}{\left(\frac{Q_H \cdot f_u \cdot CL_{int}}{Q_H + f_u \cdot CL_{int}} \right)} \quad \text{(Eq. 17)}$$

where f_a is the fraction of drug absorbed from the gastrointestinal lumen.

Interestingly, as shown in equations 16 and 17, the AUC_{PO} is independent of hepatic blood flow (Q_H), while the AUC_{IV} depends on not only $f_u \cdot CL_{int}$, but also Q_H.

To illustrate the effects of enzyme inhibition and induction on the AUCs after oral and intravenous

administration, computer simulations were carried out for high, intermediate and low clearance drugs by using equations 16 and 17 (figures 3 and 4). As shown in figure 3, a decrease in the CL_{int} caused by inhibition yields an almost proportional increase in the AUC after oral administration, regardless of whether the compound is a high or low clearance drug, while after intravenous administration a decrease in the CL_{int} affects the AUC of low clearance drugs more than that of high clearance drugs. Similarly, an increase in the CL_{int} caused by induction has significant effects on the AUC_{PO} of either high, intermediate or low clearance drugs, but only on the AUC_{IV} of low clearance drugs (fig. 4). Enzyme inhibition or induction has little effect on the AUC_{IV} of high clearance drugs, because the hepatic clearance is limited by the hepatic blood flow, as indicated in equation 14. These simulations illustrate the point that the effect of enzyme inhibition (or induction) *in vivo* depends on whether the drug to be studied is a high or low clearance drug, and on whether the drug is given orally or intravenously.

The indinavir-ketoconazole interaction is a good example that *in vivo* drug-drug interaction is route- and drug-dependent (low or high clearance drug). Indinavir is a high clearance drug with a plasma clearance of 4.8 to 5.4 L/h/kg (80 to 90 ml/min/kg) in rats and 0.9 to 1 L/h/kg (15 to 17 ml/min/kg) in patients with AIDS.[121] These values exceed rat hepatic blood flow [3.6 to 4.2 L/h/kg (60 to 70 ml/min/kg)] and are similar to human hepatic blood flow [1.2 L/h/kg (20 ml/min/kg)]. Indinavir is eliminated exclusively by CYP3A-mediated metabolism in both rats and humans.[103,122]

In vitro studies with rat and human liver microsomes indicate that ketoconazole competitively inhibited the metabolism of indinavir, with a K_i of about 0.25 μmol/L for both rat and human liver microsomes.[123] Coadministration of oral ketoconazole (25 mg/kg) had little inhibitory effect on indinavir clearance or AUC after intravenous indinavir 10 mg/kg in rats. Clearance decreased from 5.2 L/h/kg (87 ml/min/kg) in control rats to 5.0 L/h/kg (83 ml/min/kg) in ketoconazole-coadmin-

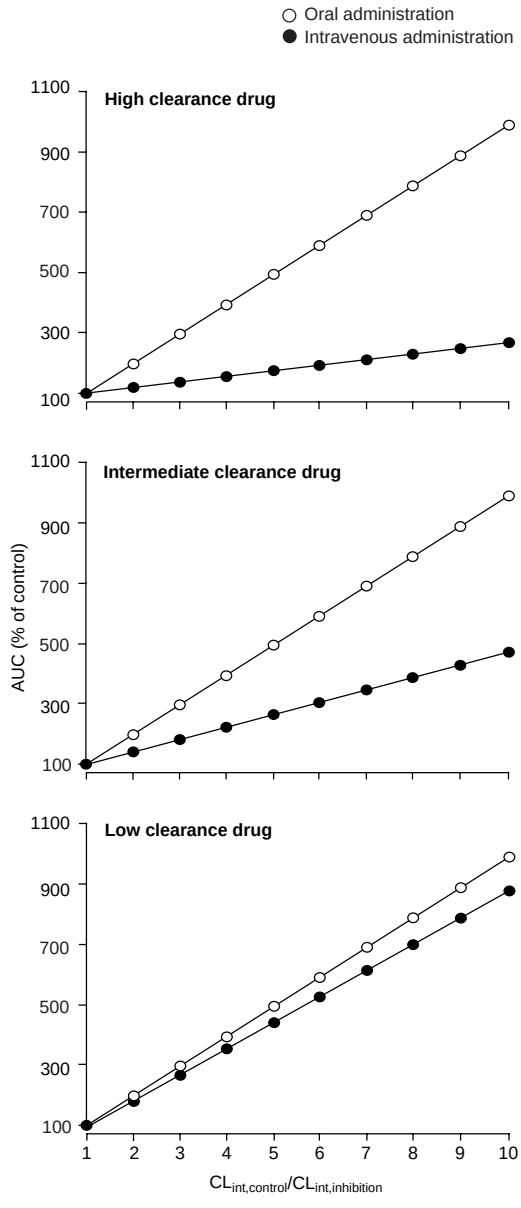

Fig. 3. Simulated effect of drug inhibition on the area under the concentration-time curve after intravenous administration and area under the concentration-time curve after oral administration of a high clearance (**top**), intermediate clearance (**middle**) and low clearance (**bottom**) drug. The value of the unbound fraction of drug in blood multiplied by the intrinsic clearance (CL_{int}) for the 3 graphs are 360, 120 and 12 L/h (6000, 2000 and 200 ml/min), respectively, and the fraction of drug absorbed from the gastrointestinal lumen is assumed to be equal to unity. The hepatic blood flow is 90 L/h (1500 ml/min).

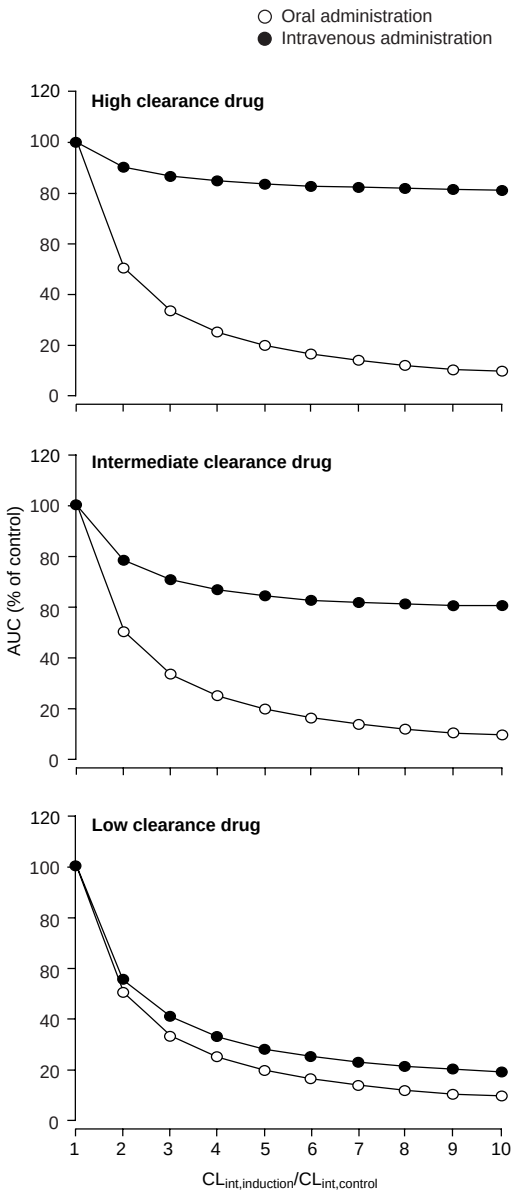

Fig. 4. Simulated effect of drug induction on the area under the concentration-time curve after intravenous administration and area under the concentration-time curve after oral administration of a high clearance (**top**), intermediate clearance (**middle**) and low clearance (**bottom**) drug. The value of the unbound fraction of drug in blood multiplied by the intrinsic clearance (CL_{int}) for the 3 graphs are 360, 120 and 12 L/h (6000, 2000 and 200 ml/min), respectively, and the fraction of drug absorbed from the gastrointestinal lumen is assumed to be equal to unity. The hepatic blood flow is 90 L/h (1500 ml/min).

istered rats. However, oral ketoconazole significantly increased the bioavailability of oral indinavir (from 20 to 89%) and its AUC.[123] Similarly, coadministration of ketoconazole (400mg orally for 4 days) increased the AUC of indinavir 400mg in healthy volunteers by approximately 62% following oral administration.[124]

On the other hand, ketoconazole is a low clearance drug with a clearance of 0.36 to 0.42 L/h/kg (6 to 7 ml/min/kg) in rats. *In vitro* studies with rat liver microsomes revealed that indinavir also competitively inhibited the metabolism of ketoconazole, with a K_i of 4.5 μmol/L. As expected, coadministration of oral indinavir 20 mg/kg in rats significantly increased the AUC of ketoconazole by 2-fold after both intravenous and oral administration of ketoconazole.[123] The clearance of ketoconazole in rats decreased from 0.51 L/h/kg when given alone to 0.27 L/h/kg when coadministered with indinavir.

As shown in equations 5 to 7, the degree of inhibition depends not only on the Km and K_i values of substrate and inhibitor, but also their concentrations, [S] and [I]. Both [S] and [I] continue to change as a function of time *in vivo* following drug administration, unless under steady state conditions. Thus, appropriate pharmacokinetic models are needed in order to obtain accurate *in vitro*/*in vivo* extrapolation. Lin et al.[125] successfully applied a physiologically based pharmacokinetic model incorporating the Km and K_i values together with the pharmacokinetic parameters of the plasma profiles of the parent drug and its metabolite to predict the quantitative effect of product inhibition of salicylamide on the elimination of ethenzamide (ethoxybenzamide) in rabbits after a single dose. However, a close examination of literature reveals that in most cases *in vitro* interaction studies were carried out to assess the potential of drug interaction, more or less in a qualitative sense, by comparing the relative affinity of the substrate (Km) and inhibitor (K_i), and their concentration ranges in clinical studies. One of the most common approaches is the use of *in vitro* K_i values together with *in vivo* values of the peak plasma concentra-

tion of inhibitor to forecast the possibility of drug-drug interaction.

Even for qualitative prediction, *in vitro*/*in vivo* extrapolation of drug-drug interaction appears to be difficult and controversial. One of the controversies is whether total (bound + unbound) or unbound plasma concentrations of inhibitor should be used to predict *in vivo* drug interaction. A basic tenet of pharmacokinetics is that only the unbound drug can diffuse across hepatocytes and that the unbound drug concentration in the blood is in equilibrium with that in the hepatocytes. Thus, it is generally believed that only unbound inhibitor can compete with substrate for the enzymes.[126-129]

However, there are reports that contradict this tenet. For example, instead of unbound inhibitor concentration, total plasma concentration of ketoconazole gave a good *in vitro*/*in vivo* extrapolation of the terfenadine-ketoconazole interaction.[130] Similarly, Tran et al.[131] reported that the *in vivo* K_i values of stiripentol on the metabolism of carbamazepine were more consistent with the *in vitro* K_i values when total plasma concentrations of stiripentol were used to estimate the *in vivo* K_i values. These investigators speculate that stiripentol concentration at the enzyme site is much higher than the unbound concentration in the blood because of a high liver/plasma partition ratio. A similar phenomenon has been described for selective serotonin reuptake inhibitors.[132,133] Good *in vitro*/*in vivo* extrapolation of drug-drug interactions with selective serotonin inhibitors was obtained only when the liver/plasma partition ratio was taken into account.

The issue of intrahepatic exposure of enzyme to inhibitor or substrate and its relationship with plasma concentration requires further investigation. Recently, factors affecting the *in vitro*/*in vivo* extrapolation of drug-drug interactions have been critically reviewed by Bertz and Granneman.[134]

Sometimes, the failure of *in vitro*/*in vivo* extrapolation may originate from the nature and design of *in vitro* experiments. Cimetidine, an H_2 receptor antagonist, has been well documented to inhibit CYP-mediated drug metabolism in humans.[5] However, the concentration of cimetidine required for *in vitro* inhibition of a CYP-mediated reaction is typically 100 to 1000 times greater than the plasma concentration of cimetidine associated with the inhibition of drug metabolism in patients.[135,136] Clearly, the *in vitro* data will falsely predict the potential *in vivo* drug interaction. Although the reason for the *in vitro* and *in vivo* discrepancy is not fully understood, recent studies by Chang et al.[137,138] have suggested that cimetidine may be a mechanism-based inhibitor. This may explain the *in vitro*/*in vivo* discrepancy.

In vitro studies with rat liver microsomes revealed that cimetidine inhibited the activity of CYP2C11, CYP2B1/2 and CYP3A1/2, with IC_{50} values in the range of 1.0 to 7.4 mmol/L.[136] Preincubation of rat liver microsomes with a low concentration (0.05 mmol/L) of cimetidine in the presence of NADPH resulted in a substantial decrease in enzyme activity, suggesting that a mechanism-based inactivation is involved.[138] It is possible that cimetidine acts as an irreversible inhibitor *in vivo*, but as a reversible inhibitor *in vitro*. Therefore, an understanding of the underlying mechanism involved in drug interaction is important in order to provide a rational basis for designing experimental conditions.

Similarly, *in vitro* studies failed to predict an *in vivo* diltiazem-lovastatin interaction. Both diltiazem and lovastatin are metabolised predominantly by CYP3A4 in humans.[139,140] Pretreatment with diltiazem 120mg twice daily for 2 weeks increased both the maximum drug concentration (C_{max}) and AUC of lovastatin by ≈4-fold.[141] These increases are greater than predicted from the average plasma concentration (<1 μmol/L) and *in vitro* K_i (>100 μmol/L) of diltiazem. Diltiazem, a calcium antagonist containing tertiary amine, is known to form MI complex upon oxidation.[142] Thus, the *in vitro*/*in vivo* discrepancy is most likely due to the nature and design of *in vitro* experiments in which diltiazem acts as a reversible competitive inhibitor while it forms MI complexation *in vivo*. Clinical studies also showed that diltiazem inhibits the metabolism of triazolam[143] and midazolam.[144]

Another important factor is the relative contribution of the metabolic fraction to the overall elimination. A significant interaction occurs only when drugs compete for the same enzyme system and when the metabolic reaction is a major elimination pathway. Rowland and Matin[145] have developed a pharmacokinetic model to evaluate the relative contribution of the metabolic fraction (f_m) on the degree of drug interaction. They concluded that a significant drug interaction occurs only when the f_m of a particular pathway being inhibited is greater than 50% of total clearance.

In addition, sources of inaccuracy in predicting *in vivo* drug interaction may include the presence of extrahepatic metabolism and active drug transport in the liver.

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.

As an example, coadministration of terfenadine, an antihistamine agent, and ketoconazole led to fatal ventricular arrhythmias in some patients.[146] Terfenadine is a widely used histamine H_1 receptor antagonist. It is metabolised extensively by CYP-3A4 in humans to form 2 metabolites by *N*-dealkylation and hydroxylation.[147] After oral administration of a 60mg 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. *In vitro* studies[148] showed that terfenadine is equipotent compared with quinidine as a blocker of the delayed rectifier potassium current which controls the duration of the QT interval. Thus, episodes of torsade de pointes observed during ketoconazole-terfenadine coadministration are most likely to result from the quinidine-like action of terfenadine that has accumulated in plasma.[149]

Clinical data showed that itraconazole[150] and erythromycin[151] 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.

In addition, drug-drug interaction can be stereoselective. Investigators should consider stereochemistry when evaluating drug interaction.

For example, warfarin, an oral anticoagulant, is marketed as a racemic mixture consisting of equal amounts of *R*- and *S*-warfarin. The pharmacologically active *S*-warfarin is eliminated almost entirely as *S*-7-hydroxy-warfarin and a small amount of *S*-6-hydroxy-warfarin in humans. In contrast, *R*-warfarin is mainly converted to *R*-6-hydroxy-warfarin and some *R*-7-hydroxy-warfarin.[152] *In vitro* studies with human liver microsomes indicate that both 6- and 7-hydroxylation of *S*-warfarin are catalysed exclusively by human CYP2C9, whereas the 6- and 7-hydroxylation of *R*-warfarin is mainly mediated by human CYP1A2 and CYP2C19.[153] Coadministration of enoxacin, a quinolone antibiotic and an inhibitor of CYP1A2, resulted in a decrease in the clearance of *R*-warfarin but not of *S*-warfarin.[154] As expected, enoxacin did not affect the hypoprothrombinaemic response produced by warfarin because this antibiotic had no effect on *S*-warfarin elimination.[154]

Similarly, cimetidine inhibited human metabolism of *R*-warfarin, while having little effect on *S*-warfarin.[155] Further studies in healthy individuals indicated that treatment with cimetidine resulted in a significant decrease in the formation of *R*-6- and *R*-7-hydroxy-warfarin, but had no effect on the

formation of *S*-6- and *S*-7-hydroxy-warfarin.[156] Thus, it is expected that cimetidine has little effect on the anticoagulant activity of warfarin.

In contrast, when administered concomitantly with warfarin, phenylbutazone caused profound potentiation of the hypoprothrombinaemic response, because phenylbutazone stereoselectively inhibited the metabolism of *S*-warfarin.[152] The potentiation of anticoagulant effect of warfarin by the antiarrhythmic agent amiodarone has also been reported.[157] Heimark et al.[158] studied the mechanism of interaction between amiodarone and warfarin and found that amiodarone decreased the total body clearance of both *R*- and *S*-warfarin in healthy individuals but not to the same degree. Amiodarone stereoselectively inhibited the metabolism of *S*-warfarin more than that of *R*-warfarin. In agreement, *in vitro* studies with human liver microsomes showed that amiodarone inhibited the metabolism of *S*-warfarin more strongly than that of *R*-warfarin.[158] These results suggest that the enhanced anticoagulant effect observed when amiodarone and warfarin are coadministered is attributed to stereoselective CYP inhibition.

Inhibition can also reduce clinical efficacy, if the drug is a prodrug requiring metabolic activation to achieve its effects and activation is blocked. Codeine is a good example, being extensively metabolised by glucuronidation, while the *O*-demethylation of codeine to morphine is a minor pathway mediated by CYP2D6.[159] Since only a small fraction of the drug is metabolised by *O*-demethylation, inhibition of CYP2D6 by other drugs will have little effect on pharmacokinetics of codeine itself. However, inhibition of CYP2D6 will have a significant effect on the formation of morphine, thus altering the analgesic efficacy of the parent drug, codeine. Since codeine is often administered with drugs that inhibit CYP2D6, this offers scope for interactions that could modulate the efficacy in patients. On the other hand, proguanil is converted through a major pathway to its active antimalarial metabolite, cycloguanil, by CYP2C19.[160] Inhibition of CYP2C19 will result in alterations of both the pharmacokinetics and therapeutic effects of this drug.

Drug interactions may relate to specific competitive inhibition of polymorphic enzymes. Omeprazole is a proton pump inhibitor used to treat peptic ulcers and reflux oesophagitis. It is mainly metabolised by CYP2C19.[161] Diazepam is also predominantly metabolised by CYP2C19.[161] The CYP2C19 isoform is known to be polymorphic, and ≈2 to 6% of Caucasians or 18 to 22% of Asians have been found to be PM.[18,19] Coadministration of omeprazole resulted in a significant increase in the AUC of diazepam in EM, but had no effect on the diazepam AUC in PMs.[161] The fact that both omeprazole and diazepam are mainly metabolised by the same enzyme, CYP2C19, explains why the 2 drugs interact in EMs, but not in PMs. In PMs, there is little or no enzyme for which diazepam and omeprazole could compete. Similarly, coadministration of quinidine, a CYP2D6 inhibitor, has been shown to increase plasma concentrations of encainide, an antiarrhythmic agent metabolised mainly by CYP2D6 in EMs, but quinidine had little effect on plasma concentrations in PMs.[162] Collectively, these results suggest that EMs are more susceptible to enzyme inhibition than PMs.

The importance of interethnic differences in drug disposition has recently been recognised. Because of ethnic differences in the representation of EMs and PMs, one racial group may be more sensitive to drug inhibition than another. For instance, the extent of inhibitory effect of omeprazole on *S*-mephenytoin and diazepam is dependent on ethnicity. The degree of inhibition was much greater in European White individuals than in Chinese people.[163,164] Although the reason for this ethnic difference in drug inhibition is not fully understood, an over-representation of the heterozygous genotype of CYP2C19 among Chinese EMs might have accounted for the observed lower drug inhibition by omeprazole. Like PMs, these heterozygous EMs appear to be less susceptible to enzyme inhibition than homozygous EMs. The proportion of heterozygotes compared with homozygous dominant EMs is about 5-fold greater in Chinese

compared than in White individuals.[21] Thus, interethnic variability should be taken into consideration when drug interaction data are extrapolated from one racial population to another group.

In addition to the reversible competitive inhibitors, a number of drugs have been shown to be irreversible CYP inhibitors via enzyme destruction or MI complexation. This class of drugs characteristically exhibits time- and dose-dependent pharmacokinetics when given orally or intravenously. L-754 394, an experimental HIV protease inhibitor, is a good example. *In vitro* microsomal studies revealed that L-754 394 is a mechanism-based inhibitor. Studies in rats, dogs and monkeys have shown that the drug exhibits time- and dose-dependent pharmacokinetics.[165] The apparent clearance decreased with increasing dose. However, the dose-dependency cannot be explained by Km. L-754 394 in plasma declined log-linearly with time, but with an apparent $t_{1/2}$ that increased with the dose. Furthermore, the apparent clearance of L-754 394 decreased after multiple doses. It is clear that inactivation of metabolic enzymes depends not only on the dose, but also on the duration and frequency of administration.

Because the pharmacokinetics of drugs that inactivate enzymes are time- and dose-dependent, drug interactions caused by enzyme inactivation will depend on the timing of the administration of enzyme inactivators and other drugs, as well as on their doses. L-754 394 and indinavir interaction is a good example. L-754 394 increased the AUC of indinavir in the plasma of rats by ≈2-fold when 10 mg/kg of each drug was given orally at the same time. However, when L-754 394 was given 1 hour before administration of indinavir, there was a 5-fold increase in the AUC of indinavir (Lin et al., unpublished data).

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 the inhibitor, and *de novo* biosynthesis of new enzymes is the only means by which activity can be restored. Clearly, clinical and pharmacokinetic consequences of irreversible drug inhibition are quite complicated, depending on the duration and frequency of administration. The long term effects of irreversible inhibition on CYP is yet unknown, and further studies need to address this question.

Metabolic drug interaction is usually regarded as potentially dangerous, or at least undesirable. However, there are times when these interactions may be exploited. For example, because these 2 drugs are substrates for the same human CYP3A4, the antifungal agent ketoconazole is used with cyclosporin, an immunosuppressive agent, to prolong the elimination of the cyclosporin.[166] The idea is to use the relatively inexpensive ketoconazole to specifically inhibit the metabolism of the very expensive cyclosporin, thereby minimising the cost of long term immunosuppressive therapy. Keogh et al.[167] have reported that ketoconazole reduced by 80% the dose of cyclosporin needed to maintain target concentrations in patients after cardiac transplantation, with a cost savings per patient of ≈$US5200 in the first year. The use of other drugs to reduce the cost of cyclosporin also has been reported.[168]

Similarly, coadministration of ritonavir, an HIV protease inhibitor, enhanced the oral absorption of saquinavir, another HIV protease inhibitor. Saquinavir has a poor bioavailability (<5%). Following a single dose of ritonavir (600mg) and saquinavir (200mg), a >50-fold increase in the plasma AUC of saquinavir was observed in volunteers.[169] The combined regimen of ritonavir and saquinavir may be of benefit in the treatment of AIDS.

5.2 Induction of CYP

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

caused by the increased production of the toxic metabolites.

Rifampicin is one of the most potent enzyme inducers known to humans. It induces several CYP isoforms, including CYP2C and CYP3A.[170,171] 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.[172] Heimark et al.[173] have shown that the reduction in hypoprothrombinaemic response of warfarin by rifampicin was caused by increased clearance of both warfarin enantiomers.

Another clinically important interaction with rifampicin involves the concomitant administration of oral contraceptives, which has been reported to result in menstrual disturbance and unplanned pregnancies. The increased metabolism of both estrogenic and progesterogenic components of oral contraceptives is believed to be the underlying mechanism.[174] A 4-fold increase in the rate of hydroxylation of estradiol and ethinylestradiol in patients treated with rifampicin was associated with an increase of CYP content in liver biopsies.[175]

Rifampicin also increases the metabolism of cyclosporin in patients, resulting in low blood concentrations of the immunosuppressive agent.[176] The subtherapeutic blood concentrations of cyclosporin caused by coadministration of rifampicin have frequently resulted in acute allograft rejection.[177,178]

Enzyme induction represents a common problem in the management of epilepsy. Phenobarbital, phenytoin and carbamazepine are potent inducers of CYP.[179] Like rifampicin, phenobarbital can stimulate the catalytic activity of several CYP isoforms, including CYP2C and CYP3A.[9] An early reported interactions with phenobarbital also involved oral anticoagulants. Concurrent administration of phenobarbital and warfarin resulted in a decrease in plasma concentrations of warfarin and in its anticoagulant effects.[180] Phenytoin and carbamazepine appear to be less potent inducers in humans than rifampicin and phenobarbital at doses used in clinical practice. Phenazone (antipyrine) clearance was increased by 60 and 90%, respectively, in healthy volunteers after multiple doses of phenytoin or carbamazepine.[181]

As with enzyme inhibition, EMs are more susceptible to enzyme induction than PMs. Treatment with rifampicin caused a substantial increase in the metabolism of *S*-mephenytoin in EMs, but had no effect on the metabolism of *S*-mephenytoin in PMs.[182] *S*-Mephenytoin is known to be metabolised exclusively by CYP2C19, and PMs are genetically deficient in CYP2C19.[21,22] Thus, the lack of rifampicin induction in PMs is simply associated with the absence of CYP2C19 enzyme. Even if the mutant enzymes related to CYP2C19 were induced by rifampicin, they would not be expected to metabolise *S*-mephenytoin.

Omeprazole induces human CYP1A2[92,93] and is metabolised predominantly by CYP2C19.[161] After an oral dose of omeprazole 40mg, the plasma AUC of omeprazole in PMs of CYP2C19 was ≈5-fold higher than that in EMs.[183,184] After 7 days of treatment with omeprazole (40 mg/day), CYP1A2 induction as measured by ^{13}C-[N3-methyl]-caffeine breath test was shown to be significant in PMs, but not in EMs.[183,184] The lack of CYP1A2 induction in EMs is associated with their lower exposure to omeprazole. This example represents another cause of differential induction between PM and EM individuals.

Like PMs, elderly individuals appear to be less sensitive than younger adults to inducers. The disposition of hexobarbital before and after rifampicin treatment was studied in young and elderly healthy volunteers.[185] Rifampicin treatment produced a differential increases in *R*-(–)-hexobarbital metabolism in young (90-fold increase) and elderly individuals (19-fold increase). Similarly, the inductive effect of cigarette smoking on propranolol was much greater in young adults than in the elderly.[186] The reduced induction of drug metabolism in the elderly also has been reported by other investigators.[187-189] The reason for the age-dependent response to inducers is not fully understood and remains to be studied.

Although enzyme induction generally reduces the pharmacological effect because of increased drug metabolism, sometimes the formed metabolite 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. Collste et al.[190] studied the effect of pentobarbital on alprenolol. The oral bioavailability of alprenolol was reduced, with the AUC being reduced by 45%. Despite these marked changes in AUC, there was only a modest reduction in the pharmacological response, measured by the inhibition of exercise-induced tachycardia. The lesser reduction in dynamic response compared with the change in AUC can be explained by the β-adrenergic receptor activity of the metabolite, 4-hydroxyalprenolol, which is as potent as the parent drug.

During concomitant administration of inducers, the reduction in drug concentration can be circumvented by increasing the drug dosage. However, if dosages are increased, there is a danger of excessive accumulation of drug when the inducer is withdrawn and enzyme activity returns to normal. An example is the severe bleeding reported when hospitalised heart attack patients treated with anticoagulants returned home and discontinued the use of phenobarbital sleeping pills.[191]

A more complex inducer-drug interaction also has been reported for alcohol and paracetamol (acetaminophen).[192] Human CYP2E1 is known to be responsible for the formation of the toxic metabolite of paracetamol, *N*-acetyl-*p*-benzoquinone imine. In addition, alcohol is known not only to inhibit but also to induce CYP2E1 enzyme activity in humans. Long term alcoholism and paracetamol ingestion, thus, presents a complex and serious problem. The time interval between the last consumption of alcohol and ingestion of paracetamol is very important. If paracetamol is taken in the morning because of a headache as a result of heavy drinking the night before, there is a high risk of hepatotoxicity. This is because the alcohol concentration is insufficient to inhibit the formation of *N*-acetyl-*p*-benzoquinone imine in the liver where CYP2E1 enzyme activity was induced, resulting in an increased formation of the toxic metabolite. However, if paracetamol and alcohol are taken at the same time, the formation of the metabolite is not expected to increase because of the opposite effects of alcohol inhibition and induction.

6. Conclusions

From the viewpoint of drug therapy, to avoid potential drug-drug interactions, it is desirable to develop new drugs that are not potent CYP inhibitors or inducers and are not readily inhibited by other drugs. In reality, drug interactions caused by mutual inhibition are almost inevitable, because CYP-mediated metabolism represents a major route of elimination of many drugs and because the same CYP enzyme can metabolise numerous drugs.

It should be emphasised that only a few drug interactions, but not all of them, are clinically significant. The clinical significance of a metabolic drug interaction will depend on the magnitude of the change in the concentration of active species (parent drug and/or metabolites) at the site of pharmacological action and the therapeutic index of the drug. The smaller the difference between toxicity and efficacy, the greater the likelihood that a drug interaction will have serious clinical consequences. Thus, careful evaluation of potential drug interactions of a new drug candidate during the early stage of drug development is essential.

To this end, carefully designed *in vitro* studies can be a valuable tool to predict potential drug interactions *in vivo*. In order to accurately predict potential metabolic drug interaction, it is necessary to know the underlying mechanisms of drug inhibition, the metabolic fate of the drug, and the enzyme involvement in each metabolic pathway. Finally, an understanding of pharmacokinetic principles will facilitate the extrapolation of *in vitro* data to the *in vivo* situation.

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Correspondence and reprints: Dr *Jiunn H. Lin*, WP42-2, Drug Metabolism, Merck Research Laboratories, West Point, PA 19486, USA.
E-mail: jiunn_lin@merck.com