DRUG METABOLISM
AND
DRUG INTERACTIONS

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CONTENTS

CLINICAL IMPLICATIONS OF GENETIC POLYMORPHISMS AND DRUG INTERACTIONS MEDIATED BY CYTOCHROME P-450 ENZYMES

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SUMMARY

Hepatic oxidation is a major drug metabolising process and is carried out by the cytochrome P-450 monooxygenase system. This system consists of a variety of isoenzymes among which the cytochromes 1A2, 2C8, 2C9/10, 2C19, 2D6, 2E1 and 3A4 are involved in the oxidative metabolism of drugs. Interindividually, large differences in capacities are found. These differences are partly due to genetic constitution (genetic polymorphism, which has been proved to exist for CYP2D6 and CYP2C19) and partly due to environmental factors, among which the administration of interfering drugs can play a major role.

KEY WORDS

drug metabolism, cytochrome P-450, liver, drug interaction, genetic polymorphism

1. INTRODUCTION

Drugs can be cleared from the body by renal clearance, metabolism or both. Although every tissue has some ability to metabolise xenobiotics, the liver is the principal organ of biotransformation. Major metabolising enzymes are cytochrome P-450 monooxygenases, epoxide hydrolase, glucuronyl-transferase, acetyl-transferase, sulphotransferase and xanthine oxidase. Hepatic metabolism usually involves oxidation (phase 1) followed by conjugation with sulphate or glucuronide (phase 2). Oxidation implies the presence of a hydroxyl group either in the parent compound (hydroxylation reactions) or in a leaving group (demethylation reactions). Oxidation reactions of drugs are facilitated by the microsomal mixed-function oxidase system of which cytochrome P-450 monooxygenase is the pricipal enzyme system. Cytochrome P-450 monooxygenase consists of two protein components, a haemoprotein called cytochrome P-450 (CYP) and a flavoprotein called NADPH-cytochrome P-450 reductase /1/. CYP is the substrate- and oxygen-binding site of the enzyme system, while the reductase serves as the electron donor. CYPs are responsible for the oxidation of endogenous compounds such as steroids, fatty acids, prostaglandins, leukotrienes and biogenic amines, and of innumerable

exogenous compounds including a variety of drugs. Certain CYPs have specific functions, whereas others are non-specific. Some of the CYPs are more sensitive to interactions caused by drugs or environmental factors than others.

Since the introduction of the Selective Serotonin Reuptake Inhibitors (SSRIs) as antidepressant drugs (for example, fluoxetine and paroxetine), much effort has been directed towards cytochrome P-450 research because of the high drug interaction potential of this class of drugs /2/. At present, drug regulation authorities require adequate information on metabolic pathways and drug interaction potential for every new drug that is to be licensed.

Because of the many functions and associations of the CYPs, this paper emphasizes the genetic variation of CYP-mediated oxidation of drugs as well as the factors that affect the metabolising capacity of the CYPs.

2. GENETIC POLYMORPHISM

Genetic polymorphism of drug metabolism refers to a genetically determined variability in metabolising capacity among humans. It follows that in a population subgroups exist who differ in their metabolic capacity and ability to metabolise drugs specifically metabolised by these enzymes. Polymorphism is caused by mutations in the gene coding for the specific enzyme. These mutations can lead to enzymes with altered capacity or to the absence of the enzymes. Genetic polymorphism has been demonstrated for CYP2C19 and CYP2D6 and two phenotypes have been identified: slow (poor) metabolisers (PM) and normal (extensive) metabolisers (EM). In the case of CYP2D6 a group of ultra-rapid metabolisers (URM) has also been identified /3/. URM are the result of the presence of multiple genes for the particular enzyme resulting in the production of high concentrations of the enzyme /3/.

The pharmacokinetic and pharmacodynamic consequences of the activity of a polymorphic enzyme depend upon whether it mediates the metabolism of a parent drug into either an active or an inactive metabolite, or mediates the metabolism of an inactive prodrug into an active metabolite. In a PM, the overall clearance of the parent drug is reduced and it will accumulate in the body possibly resulting in serious toxicity unless the dose of the drug is adjusted downwards. On

the other hand, a PM can be unable to convert an inactive prodrug into the active form and thus suffer from either toxicity of the prodrug or lack of activity of the drug treatment or both. In this case, the prodrug must not be given to the PM at all, but an alternative drug that needs no conversion into an active form by the affected CYP must be found. Because of their rapid metabolism, URMs need extremely high doses of a drug metabolised by the particular enzyme in order to produce adequate serum concentrations and clinical efficacy. To be of clinical relevance, (a) the polymorphic pathway needs to represent a major contribution to the clearance of the drug of interest, (b) the drug or the prodrug must have a narrow therapeutic window, and (c) there must be little pharmacodynamic variability. When a drug can be easily titrated by direct clinical monitoring (as is the case with blood-pressure lowering drugs, for example), one need not worry about pharmacogenetics. When the clinical effects of a drug are not easily measured (as is the case with tricyclic antidepressants) *a priori* identification of a PM can avoid serious intoxications.

Identification of a PM can be made by genotyping (DNA-analysis) and by phenotyping. Phenotyping can be carried out by administering probe substances to volunteers or patients. The ideal probe substance is metabolised exclusively by the CYP enzyme of interest and the amount of metabolite formed and excreted in the urine can be analysed. In practice a probe substance is administered before bedtime and the first urine the next day is collected. The amounts of parent drug and metabolite in the urine are determined and the ratio parent drug/metabolite is calculated (metabolic ratio, a pharmacokinetic parameter indicating the *in vivo* metabolic capacity). PMs have a higher metabolic ratio than EMs. Based on population studies, a ratio has been identified above which a subject is identified as a PM. When the frequency is plotted against the metabolic ratio, the resulting graphic display yields two or more subgroups if genetic polymorphism is present within the test population (see Figure 1). In this way, a 'cocktail' of probe substances has been used succesfully to characterise the individual metabolic capacity of several CYP enzymes in one study /4/.

Some drugs are capable of inhibiting the capacity of a CYP and thus converting a genotypic EM into a phenotypic PM. For example, quinidine in very low (subtherapeutic) dosages effectively inhibits CYP2D6. Applying this drug to an EM will result in enzyme

inhibition and reduced metabolism of co-administered drugs that are dependent on this specific enzyme for their metabolic clearance or activation. In human drug studies, this phenomenon is sometimes used to study poor metaboliser drug kinetics in genotypic EMs when there is a lack of genotypic PMs. Administering these enzyme inhibiting drugs to genotypic PMs usually has no further inhibitory effects.

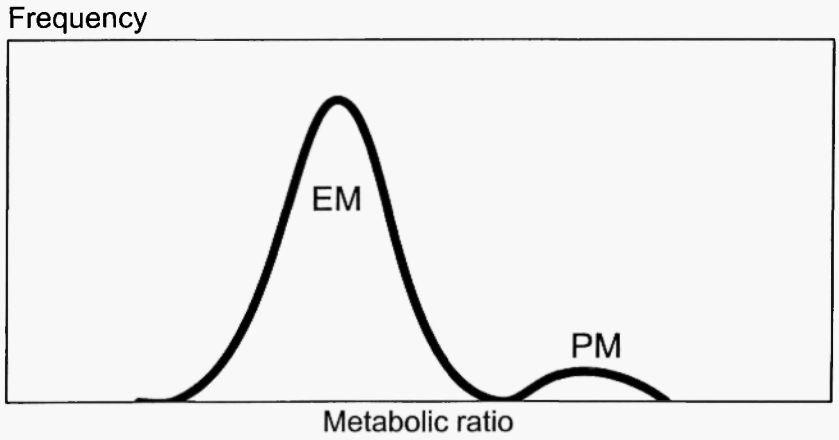

Fig. 1: Schematic representation of the frequency of the metabolic ratio in a population. EM: extensive metaboliser, PM: poor metaboliser.

3. CYTOCHROME P-450 ENZYMES OF RELEVANCE FOR DRUG METABOLISM

The majority of human cytochromes P-450 involved in drug metabolism can be divided into three gene families, CYP1, CYP2 and CYP3. These enzyme families share at least 40% amino acid homology. Each family can be divided into several subfamilies (designated by a capital letter, e.g. CYP2B, CYP2C, etc.). The different members of these subfamilies share at least 55% amino acid homology. Within the subfamilies the individual enzymes (isoenzymes) are identified with an arabic number. At present, more than 50 different cytochrome P-450 enzymes have been identified of which at least 15 are known to be involved in human drug metabolism. Of these 15 isoforms seven account for the metabolism of the majority of the drugs that are commonly used. These isoenzymes include CYP1A2, CYP2C8, CYP2C9/10, CYP2C19, CYP2D6, CYP2E1 and

CYP3A4. Quantitatively, CYP3A4 is the most important CYP enzyme in the liver, followed by CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP2C19. Metabolism of drugs usually takes place in the liver, but in the case of drugs metabolised by CYP3A4 it also takes place in the gut wall which can lead to extensive presystemic metabolism. There is increasing evidence that metabolism by CYPs also takes place in the lungs and in the kidneys. The metabolising capacity of the CYP enzymes shows wide interindividual differences, partly caused by genetic factors (CYP2C19 and CYP2D6), partly caused by gender or ethnicity, partly caused by disease states and partly caused by interactions.

Drug-drug interactions at the level of metabolism (biotransformation) can principally occur through enzyme induction and through enzyme inhibition. Enzyme induction is defined as increased enzyme activity caused by an increase of the biosynthesis of enzymes; it is often caused by exogenous factors (drugs, chemicals, smoking) and may be selective. It requires time before the inducing effect is maximal and is dependent on the dose of the inducing agent. The effect is usually reversible. Some well-known inducers of hepatic and other metabolism are cigarette smoke, rifampicin, phenytoin, phenobarbitone, carbamazepine and ethanol.

Since one CYP may bind many different substrates, competition at the site of drug binding by cosubstrates and by other substrates or chemicals may cause inhibition. Enzyme inhibition is defined as decreased enzyme activity caused by competition of substrates at the enzyme level. It occurs acutely on simultaneous administration of compounds, is dose dependent, most often selective and in general reversible. Some well-known potent enzyme inhibitors are cimetidine (aselective), quinidine, paroxetine, fluoxetine, fluvoxamine, imidazoles, and macrolides.

Some CYP enzymes are extremely sensitive to either induction or inhibition by food constituents, environmental factors or drugs (see Table 1).

In vivo investigation of the metabolism of drugs is complicated by the fact that metabolism seldom takes place through one exclusive enzymatic pathway. In general, hydroxylation of most drugs is predominantly mediated by CYP2D6 and demethylation predominantly by CYP1A2, CYP2C and CYP3A4.

At least 15 different isoenzymes have been characterised to varying degrees in the human liver. In this section, the seven major CYP isoenzymes involved in human drug metabolism are discussed with their major substrates and drug-drug interactions.

TABLE 1

Overview of cytochrome p450 enzymes (CYP) of relevance for human drug metabolism with inhibitors and inducers

CYP	inhibited by	induced by
1A2	fluvoxamine, caffeine, moclobemide, diltiazem, enoxacin, ciprofloxacin, pefloxacin	omeprazole, cigarette smoke
2C9	isoniazid	rifampicin, omeprazole
2C19	omeprazole, fluoxetine, moclobemide, proguanil	rifampicin, phenobarbitone
2D6	quinidine, paroxetine, fluoxetine, sertraline, moclobemide, cimetidine	rifampicin, phenobarbitone
2E1	disulfiram, isoniazid	ethanol, isoniazid
3A4	cimetidine, ketoconazole, itraconazole, fluconazole, nefazodone, sertraline, isoniazid, diltiazem, grapefruit juice, erythromycin	rifampicin, carbamazepine, phenobarbitone, pentobarbitone, phenytoin

3.1. Cytochrome P-450 2D6

CYP2D6 is the best understood CYP isoenzyme /5/. It is a member of the CYP2 family which contains the largest number of CYPs. The CYP2D6 gene is located on chromosome 22. Two other CYP genes (CYP2D7 and 2D8) are located upstream. These genes are defective and not expressed. CYP2D6 displays polymorphism. Mutations 2D6-A, 2D6-B and 2D6-D lead to genotypic and phenotypic PMs and mutation 2D6-C leads to an enzyme with a slightly lower activity but not to a phenotypic PM /5/. The mutated alleles are recessive, so PMs are homozygous for the mutated allele and EMs comprise hetero-

zygous and homozygous dominants. Knowledge of the mutations has led to the development of polymerase chain reaction (PCR) tests enabling direct genotyping in epidemiological studies.

CYP2D6 metabolises many substrates (Table 2) including antidepressants, antipsychotics, beta-blockers and a variety of other drugs. Debrisoquine, sparteine, dextromethorphan and phenformin are used as probe drugs and are commonly used to investigate CYP2D6 polymorphism. Across all populations studied, 2-10% of individuals are PM for these probe drugs. The percentage is the highest for Whites (between 5-10% PM), followed by Afro-Americans (6%) and the lowest for Asians (0-0.7%).

TABLE 2

Drugs that are metabolised by the enzyme cytochrome P450 2D6

antiarrhythmics	sparteine, propaphenone, encainide, flecainide, mexiletine, lignocaine*, quinidine*
beta-blockers	alprenolol, metoprolol, timolol, propranolol*
antihypertensives	debrisoquine
antipsychotics	perphenazine, thioridazine, fluphenazine, haloperidol, zuclopentixol, risperidone, clozapine*
antidepressants	nortriptyline, desipramine, fluoxetine, paroxetine, desmethylcitalopram, sertraline, brofaromine, mianserin, maprotiline, imipramine* (hydroxylation), amitriptyline* (demethylation and hydroxylation), clomipramine* (hydroxylation), fluvoxamine*
amphetamines	4-methoxyamphetamine, methylenedioxymethamphetamine (ecstasy)
analgesics	tramadol, hydrocodone, dihydrococaine, codeine* (conversion to morphine)
others	tropisetrone, captopril, phenphormine, vinblastine, loratadine*, ritonavir*

*Other systems are involved in the clearance of the drug. A reduced capacity therefore has limited consequences for total body clearance.

The clinical consequences of PM have been studied extensively. It has been suggested that limited CYP2D6 oxidation capacity might be associated with the development of Parkinson's disease, hepatitis and certain forms of cancer /6/. These implications have never been substantiated. However, it has been established that limited oxidation capacity may have important consequences for the oxidative clearance of drugs. Some examples are outlined below.

Extensive pharmacokinetic research has been carried out with the tricyclic antidepressants as prototype drugs for CYP metabolism. Desipramine is oxidised mainly by CYP2D6 to 2-hydroxydesipramine /7/. EMs have an average half-life for desipramine of 17 hours in contrast to PMs who have an average half-life of desipramine of more than 100 hours /8/. This illustrates the high specificity of CYP2D6 for desipramine. Therefore, desipramine can also be used as a probe drug for this enzyme. Since high desipramine serum levels are potentially cardiotoxic, it is obvious that in the case of a poor metaboliser, the dose has to be reduced. These observations are also true for the antidepressant nortriptyline /9/, the antipsychotic drugs zuclopentixol /10/, perphenazine /11/ and haloperidol /12/, and for the beta-blocking drugs metoprolol /13/ and timolol /14/.

The tricyclic antidepressant clomipramine is demethylated to the pharmacologically active desmethylclomipramine by CYP2C19, and both clomipramine and desmethylclomipramine are hydroxylated to the corresponding hydroxy-metabolites by CYP2D6 /15,16/. Poor metabolism of CYP2D6 will result in accumulation of both clomipramine and desmethylclomipramine and will necessitate a reduction of the dose to avoid toxicity.

The antidepressant paroxetine is metabolised by CYP2D6 and also by a minor parallel route. Paroxetine is also a strong inhibitor of CYP2D6 and therefore inhibits its own metabolism, making the parallel route the most important one for oxidation /17,18/ When single-dose pharmacokinetics of paroxetine are studied, there is a large difference in paroxetine kinetics between genotypic PMs and EMs. EMs have a 25-fold higher clearance of paroxetine than PMs. With chronic dosing, however, there is only a 2-fold difference in clearance between EMs and PMs. This is caused by the auto-inhibition of CYP2D6 that occurs during chronic treatment with paroxetine. Due to this phenomenon, PMs can be treated with the same paroxetine dosage as EMs /19/.

The antipsychotic drug risperidone is hydroxylated to the pharmacologically active hydroxyrisperidone by CYP2D6 /20,21/. Both compounds are further metabolised in a CYP2D6 independent way. Poor metabolism will result in higher risperidone levels and reduced hydroxyrisperidone levels. Since both compounds are equally active, PM will not result in a higher antipsychotic moiety and doses for PMs are the same as for EMs.

Codeine is metabolised by CYP2D6 to morphine which has the analgesic properties of the drug /22/. Poor metabolism of CYP2D6 results in the absence of the analgesic effects of codeine.

CYP2D6 has a high affinity for substrates and limited capacity and is very sensitive to enzyme inhibition. Potent inhibitors of the enzyme are quinidine, paroxetine and fluoxetine. These drugs will change a genotypic extensive metaboliser into a phenotypic poor metaboliser /23/. When these drugs are combined with any drug shown in Table 2, the latter drug will accumulate in the body, and lead either to toxicity or, in the case of a prodrug that needs to be metabolised to its active form, in a lack of effect, or both. Such serious drug-drug interactions have been reported, for example, with amitriptyline, desipramine, alprazolam, metoprolol and propranolol /24-28/. There are other drugs that inhibit CYP2D6 in a more concentration dependent way. Sertraline, for example, usually has hardly any inhibiting effect on CYP2D6 when the drug is administered at relatively low doses (50 mg/day) but there is a marked inhibitory effect at higher doses (>100 mg/day) /29/. At these higher doses drug-drug interactions are likely to occur /30/.

CYP2D6 does not seem to be very sensitive to enzyme induction. The classical hepatic enzyme inducing drug rifampicin leads only to a marginal increase of the metabolic ratio in EMs for CYP2D6 /31/, as does phenobarbitone /32/, but not pentobarbitone /33/. At present there are no examples of drugs that are capable of markedly inducing the effect of CYP2D6.

3.2. Cytochrome P-450 2C

The cytochrome P-450 2C subfamily consists of several closely related enzymes and the genes coding for them. Among others, CYP2C8, CYP2C9, CYP2C10, and CYP2C19 have been identified /5/. CYP2C9 and CYP2C10 are closely related and differ by only two amino acids. The stereospecific 4′-hydroxylation of (S)-mephenytoin

shows polymorphism and the best candidate gene as the target of the mephenytoin polymorphism is the CYP2C19 gene. About 2-5% of Whites are poor metabolisers for CYP2C19; 18-25% of Afro-Americans and Asians are PMs.

The CYP2C family metabolises several substrates (Table 3). The clinical consequences of impaired metabolism by this enzyme system have been less well studied than those of CYP2D6. Some relevant examples are summarised below.

TABLE 3

Overview of the cytochrome P450 2C subfamily with the drugs that are metabolised by different members of this family

enzyme	substrate
CYP2C8	paclitaxel*, tolbutamide*, warfarin*
CYP2C9/10	diclofenac, dicoumarol, ibuprofen, naproxen, tamoxifen, tenoxicam, carbamazepine*, phenytoin*, warfarin*
CYP2C19	citalopram, indomethacin, papaverine, proguanil, teniposide, clomipramine* (demethylation), diazepam*, hexobarbitone*, imipramine* (demethylation), moclobemide*, omeprazole*, phenytoin*, propranolol*, tolbutamide*, warfarin*

* Other systems are involved in the clearance of the drug. A reduced capacity therefore has limited consequences for total body clearance.

The antimalaria prodrug proguanil is activated to the active cycloguanil by CYP2C19 /34/. EMs excrete more cycloguanil than PMs do (5.8% vs 1.6%) /35/. It is to be expected that in populations with a high percentage of PMs insufficient cycloguanil would be formed and that antimalarial treatment is likely to be ineffective.

Omeprazole (proton pump inhibitor) is metabolised to omeprazol-sulphone and hydroxy-omeprazole by CYP2C19 /36/. Omeprazole is usually rapidly cleared fom the body but PMs of S-mephenytoin have higher AUCs and longer half-lifes of omeprazole /37/. However

omeprazole is not a reversible receptor antagonist but binds irreversibly. Despite its rapid clearance in EMs it has a long duration of action and large interindividual pharmacokinetic differences will thus have only limited clinical consequences. Because of its selective metabolism by CYP2C19, omeprazole is considered as a test substance for this isoenzyme by some researchers.

CYP2C19 is not very sensitive to inhibition and induction. Omeprazole, for example, is an inhibitor of CYP2C19 /38/. During chronic administration of this drug, it inhibits its own metabolism, thus reducing any diferences in omeprazole pharmacokinetics between PMs and EMs. In combination with omeprazole, diazepam clearance is reduced by 25-38% /39/.

CYP2C9 is inhibited by fluoxetine as illustrated by reported interactions with phenytoin /40/. Because phenytoin shows nonlinear pharmacokinetics in the therapeutic range (i.e. a small increase of dose results in a disproportionally large increase of serum concentration) an interaction with a drug such as fluoxetine that has only limited influence on phenytoin clearance may become clinically very relevant.

3.3. Cytochrome P-450 3A4

CYP3A4 is the most abundant P-450 isoenzyme in the body. It is present in the gut wall and in the liver. Wide variability in CYP3A4 activity among patients and volunteers has been shown, but there is no genetic polymorphism. CYP3A4 metabolises a wide variety of drugs among which are the dihydropyridine calcium entry blockers, the benzodiazepines alprazolam, triazolam and midazolam, cyclosporin and many others (see Table 4). Metabolism in the gut by CYP3A4 is important for the bioavailability of some drugs shown in Table 4 and for conversion of the cardiotoxic prodrug terfenadine into the antihistaminergic drug terfenadinecarboxylate. CYP3A4 is very sensitive to inhibition and induction.

Potent inhibitors of CYP3A4 are nefazodone, the azoles ketoconazole, itraconazole and fluconazole, and the macrolide antibiotic erythromycin.

Ketoconazole, itraconazole and fluconazole are capable of inhibiting the metabolism of other drugs that are metabolised by CYP3A4. This is not unexpected, because the mechanism of action of these drugs as antifungals is CYP-inhibition of the fungal steroid metabolism. Ketoconazole and itraconazole are capable of inhibiting the

TABLE 4

Drugs that are metabolised by the enzyme cytochrome P450 3A4

alfentanil, alprazolam, astemizole, budesonide, cisapride, clarithromycin, cocaine, cyclophosphamide, cyclosporin, diltiazem, erythromycin, ethosuximide, ethinyl-oestradiol, felodipine, gestodene, hydrocortisone, ifosfamide, indinavir, ketoconazole, lansoprazole, lignocaine, lovastatine, midazolam, nifedipine, nimodipine, nisoldipine, nitrendipine, rapamycin, saquinavir, sertindole, sulphamethoxazole, sufentanil, terfenadine, testosterone, triazolam, troleandromycin, verapamil, zolpidem, amiodarone*, carbamazepine*, clozapine*, codeine*, caffeine*, dapsone*, dextromethorphan*, 17-beta-oestradiol*, imipramine*, quinidine*, omeprazole*, paclitaxel*, ritonavir*, tamoxifen*, warfarin*

* Other systems are involved in the clearance of the drug. A reduced capacity therefore has limited consequences for total body clearance.

presystemic, 'first-pass' and systemic metabolism of terfenadine by CYP3A4 resulting in increased concentrations of terfenadine and life-threatening cardiotoxicity has occurred /41,42/. This was the first indication to beware of the interaction potential of the azoles.

Ketoconazole and itraconazole also inhibit the presystemic and 'first pass' metabolism and systemic clearance of midazolam. This leads to an increase of the maximum serum concentration of orally administered midazolam and an increase of its elimination half-life from 3 to 8 hours. This results in a 15-fold increase of the area under the curve (AUC) of midazolam /43/, with the risk of extreme sedation of the patient. Serious interactions between ketoconazole and triazolam /44/, alprazolam /45/, and cyclosporin /46/ have also been described. These interactions result from the same mechanism as for terfenadine and midazolam. The combination of the prokinetic drug cisapride with ketoconazole, fluconazole, miconazole or itraconazole will result in serious cardiotoxicity due to inhibition of the metabolism of cisapride and its accumulation /47/.

The macrolide antibiotic erythromycin is also metabolised by CYP3A4. At first it induces this enzyme and subsequently a stable iron-erythromycin metabolite complex is formed leading to inactivation of the enzyme /48/. Due to sterical hindrance, the other macrolide antibiotics, clarithromycin, roxithromycin and azithro-

mycin, interact less with CYP3A4 than erythromycin /48/. Erythromycin can thus increase terfenadine bioavailability resulting in life-threatening cardiotoxicity as has happened with ketoconazole and itraconazole. An interaction between erythromycin and theophylline is dose dependent with relevant inhibition of theophylline clearance (by >40%) at erythromycin dosages of >1.5 g/day /48/. At the moment it is unclear whether this interaction is mediated via CYP3A4 or CYP1A2.

An unexpected finding is the inhibitory capacity of grapefruit juice on CYP3A4. Grapefruit juice inhibits the 'first-pass' metabolism of the dihydropyridine calcium entry blockers, nifedipine, felodipine, nitrendipine and nisoldipine, mediated by CYP3A4, leading to higher serum concentrations and increased effects of these drugs /49,50/. In addition, the bioavailability of oral cyclosporin and of unchanged terfenadine are markedly increased when combined with grapefruit juice /51,52/. Grapefruit juice did not influence the pharmacokinetics of intravenously administered midazolam, but gave a 105% increase in maximum serum concentration after oral administration of midazolam /53/. This indicates that grapefruit juice inhibits only the gut CYP3A4 leading to decreased presystemic metabolism but does not affect the liver CYP3A4 and the total body clearance of drugs cleared by CYP3A4 in the liver. It has been suggested that the flavonoid naringin (in the form of its aglucon naringenin) and probably also quercetin and kaempferole are the active CYP 3A4 inhibiting components /54/.

CYP3A4 can be induced by several drugs. Rifampicin is the most potent inducer of CYP3A4 known. In the literature many interactions with rifampicin are described /55/. When rifampicin is administered with oral midazolam, a 94% decrease of oral bioavailability of midazolam is observed together with a decrease of the elimination half-life from 3.1 to 1.3 hours /56/. The decreased oral bioavailability is due to induction of the gut CYP3A4 leading to increased presystemic metabolism and the decreased half-life is due to induction of the liver CYP3A4 leading to increased total body clearance. Combination of rifampicin with midazolam and most of the other drugs shown in Table 4 makes oral administration of these drugs less effective. Omeprazole also induces CYP3A4 leading to a 30% increase of the enzyme concentration /57,58/. This leads to a moderate increase of the

clearance of the drugs metabolised by CYP3A enzymes but in practice does not lead to clinical problems.

3.4. Cytochrome P-450 1A2

Cytochrome P-450 1A2 (CYP1A2) is a member of the CYP1 family together with CYP1A1. CYP1A1 is found in the placenta and in the lungs and is not a major hepatic CYP enzyme. CYP1A2 is found only in the liver and plays a major role in the metabolism of caffeine to 3-desmethylcaffeine/59/, theophylline /60/ and the antipsychotic drug clozapine /61/ (see Table 5). CYP1A2 is one of several enzymes that demethylate tertiary amines (amitriptyline, clomipramine, imipramine) to their secondary amines. Caffeine is frequently used as a test substrate for CYP1A2 activity. No genetic polymorphism for CYP1A2 has been found, although earlier research suggested a bimodal distribution of caffeine metabolism /62/. Males are reported to have a higher activity of CYP1A2 (determined by caffeine test) than females /62/. Two population studies have demonstrated that males have relatively lower serum concentrations of clozapine than females /63,64/, which is in line with these observations.

TABLE 5

Drugs that are metabolised by the enzyme cytochrome P450 1A2

cimetidine, ondansetron, phenacetin, theophylline, amiodarone*, clozapine*, caffeine*, 17-beta-oestradiol*, fluvoxamine*, imipramine*, paracetamol (acetaminophen)*, tamoxifen*, warfarin*

* Other systems are involved in the clearance of the drug. A reduced capacity therefore has limited consequences for total body clearance.

CYP1A2 is very sensitive to inhibition and induction. The most potent inhibitor known is the SSRI fluvoxamine. Interactions are to be expected when fluvoxamine is combined with theophylline and with clozapine. In the case of combination of fluvoxamine with theophylline, the theophylline serum concentration is increased nearly 3-fold which may lead to toxicity /65,66/, and in the case of combination of fluvoxamine with clozapine, the clozapine serum concen-

tration is increased nearly 5-fold /67/. Other drug-drug interactions with fluvoxamine that have been reported are reduced demethylation of the tricyclic antidepressants clomipramine, amitriptyline and imipramine /68,69/, and reduced clearance of haloperidol /70/ and methadone /71/.

Caffeine also inhibits CYP1A2 metabolism. High doses have been shown to impair CYP1A2 metabolism as illustrated by a patient who had a clozapine level of 1.500 mg/l when taking 1,200 mg caffeine daily and had a clozapine level of 0.630 mg/l when taking no caffeine /72/.

Some of the fluorquinolone antibiotics are capable of inhibiting CYP2A1 mediated metabolism. Drug-drug interaction studies have demonstrated that several fluorquinolones can inhibit theophylline clearance. Enoxacin is the most potent inhibitor of theophylline clearance (reduction of 43-74%), followed by ciprofloxacin and pefloxacin (reduction of 18-32%) and norfloxacin and ofloxacin (reduction of 3-15%) /73/. A further indication that this interaction is mediated via CYP1A2 is the fact that enoxacin and ciprofloxacin also inhibit caffeine clearance /73/.

Omeprazole induces a 30% increase of CYP1A enzymes /57,58/, and is capable of inducing the metabolism of drugs metabolised by this enzyme. In clinical practice, however, this interaction is not very relevant. It has been suggested that CYP1A2 is involved in the bioactivation of procarcinogens and that induction by omeprazol would lead to an increased risk for cancer /74/. However, there is no clinical proof for this suggestion.

An important inducer of CYP1A2 is cigarette smoke /62/. Cigarette smokers who are treated with theophylline need a much (2-fold) higher dose to achieve comparable serum concentrations of theo- phylline as do non-smokers and it is likely that the same is true for other substrates of CYP1A2.

3.5. Cytochrome P-450 2E1

The role of CYP2E1 in drug metabolism is becoming increasingly apparent. There is evidence that CYP2E1 is involved in the metabolism of paracetamol (acetaminophen), and chlorinated and fluorinated hydrocarbons, such as the volatile anaesthetics enflurane, sevoflurane, methoxyflurane and isoflurane (Table 6). In addition chlorzoxazone is selectively metabolised by CYP2E1 to 6-hydroxy-

chlorzoxazone and this drug can serve as a test substrate. CYP2E1 does not display polymorphism. CYP2E1 is sensitive to inhibition and induction (Table 1). One example of an inhibitor is disulfiram /75/. Disulfiram is used clinically as an inhibitor of the ethanol degradation pathway in alcohol addiction programmes because of the nausea it gives when ethanol is still consumed.

TABLE 6

Drugs that are metabolised by the enzyme cytochrome P450 2E1

chlorzoxazone, ethanol, enflurane, halothane, sevoflurane, methoxyflurane, isoflurane, dapsone*, paracetamol (acetaminophen)*, caffeine*

* Other systems are involved in the clearance of the drug. A reduced capacity therefore has limited consequences for total body clearance.

Isoniazid has a complex interaction profile with CYP2C9, CYP3A3/4 and CYP2E1. Interactions have been reported with phenytoin (possibly CYP2C9), carbamazepine (CYP3A3/4) and with paracetamol (acetaminophen) (CYP2E1) /76,77/. In the case of isoniazid's interaction with CYP2E1, besides inhibition there is also induction of this enzyme. Primarily isoniazid induces CYP2E1 but as long as isoniazid is present, CYP2E1 is inhibited by isoniazid. When isoniazid is eliminated, the induction of the enzyme predominates with increased oxidation of drugs metabolised by CYP2E1. Paracetamol is metabolised by CYP2E1 to the hepatotoxic metabolite N-acetyl-p-benzoquinonimine (NAPQI). When a patient who is being treated with isoniazid intoxicates himself with paracetamol, there will be increased levels of the hepatotoxic NAPQI with a marked risk for fatal hepatotoxicity /78/. This toxicity can be avoided when treatment with N-acetylcysteine is started within 24 hours after the ingestion of the paracetamol. Usually, treatment with N-acetylcysteine is started after at least 10 grams of paracetamol have been ingested (adult toxic dose). However, isoniazid and other inducers of CYP2E1 necessitate the decision to treat with N-acetylcysteine to avoid hepatotoxicity at reduced ingestions of paracetamol, from 5 grams, which is close to the regular maximum daily dose (4 grams).

4. GENDER, AGE AND RACIAL DIFFERENCES IN HEPATIC METABOLISM

The 1977 FDA guidelines excluded women with reproductive capacity from phase I and phase II drug studies /79/. Instead, women were included in studies after the efficacy of a compound was established and after teratogenicity studies were completed. This was driven by the tragedies surrounding thalidomide and diethylstilboestrol and the need to avoid such situations in the future. However, safety pharmacokinetics are determined in phase I and phase II studies. Thus information about pharmacokinetics in women and the possible need to differentiate the dose in women is not available.

As mentioned in the introduction, CYPs are responsible for the oxidation of endogenous compounds such as steroids. Since steroids may impair enzymatic capacity, women of reproductive age using hormonal contraceptives may be at risk of developing drug interactions with these steroid hormones. One important interaction is the interaction of enzyme inducing drugs such as rifampicin or carbamazepine with oestrogens. These and other combinations that induce the metabolism of oestrogens will lead to unreliable hormonal contraception. Recently the US National Institutes of Health (NIH) has recommended researchers to include women of all age groups in the study of the pharmacokinetics of new drugs with respect to (a) menstrual cycle and menopause, and (b) the influence of and the influence on hormonal contraception /79/.

There are indications of a gender-dependent metabolic capacity of CYPs /80/. Caffeine metabolism is lower in women than in men /62/, and in the population study on clozapine mentioned above, women had on average an 80% higher serum concentration of clozapine than men treated with the same dose /64/. Caffeine and clozapine are both predominantly metabolised by CYP1A2 and these results are suggestive of a gender-dependent capacity of CYP1A2. There is increasing evidence that CYP3A4 also has a gender-dependent capacity. Women appear to metabolise erythromycin, midazolam and verapamil (all substrates of CYP3A4) faster than men do /80/. For CYP2D6 and CYP2C19, the results have been less conclusive. From combined data it appears that CYP2C19 activity is higher in men than in women and for CYP2D6 there seems to be no gender difference /80/. A large population study of the pharmacokinetics of clomipramine (which is predominantly cleared by CYP2D6) has shown that there are no

differences in clomipramine pharmacokinetics between men and women of all ages /81/. In conclusion, some CYPs seem to have different metabolic capacities between men and women. However, when pharmacokinetics in women are studied and differences with men are found, one must realise that in cases when oral hormonal contraceptives are used, these are also metabolised by CYP enzymes and possibly lead to either enzyme induction or enzyme inhibition. Results from these studies need to be interpreted carefully.

There are indications that age has an influence on the capacity of CYP1A2. A large population study showed a sharp decrease in the metabolism of clozapine above the age of 55, thus suggesting a decreased capacity of CYP1A2 /64/. In contrast, a study on clomipramine (CYP2D6) showed a sharp decrease in clearance above the age of 65 /81/. Metabolic capacity will vary with hepatic blood flow, but this dissociation of metabolic capacity with age is suggestive of differing effects of ageing on the capacity of CYP1A2 and CYP2D6.

The frequencies of PMs for CYP2D6 and 2C19 have been studied in a variety of ethnically different populations and ethnic differences in the incidence of the phenotypes are becoming apparent. In nearly all populations studied, the oxidative metabolism of the probe drugs used is controlled by a single gene. However, there are some populations in which there appears to be some dissociation. In Ghanaians, for example, PMs for debrisoquine and phenformin are the same subjects, but these appear to be normal metabolisers for sparteine /82/. It thus appears that in Ghanaians sparteine is metabolised by a different enzyme than debrisoquine and phenformin.

There are also indications of racial differences in CYP2C19 specificity. The demethylation of diazepam correlates primarily with S-mephenytoin metabolism /83/, and to a lesser extent with one of the CYP3A enzymes /84,85/. White European PMs of S-mephenytoin have a diazepam half-life of 88 h and EMs have a half-life of 41 h, suggestive of control by CYP2C19 /83/. In Korean subjects, diazepam demethylation co-seggregates with the S-mephenytoin hydroxylation and is therefore also likely to be under the control of CYP2C19 /86/. Chinese PMs and EMs, however, have the same half-life for diazepam (88 h and 85 h, respectively) /87/. This is unexpected because this would mean that in the Chinese population, in contrast to other populations, diazepam demethylation is not primarily regulated by CYP2C19. This could be explained, for example, by assuming that

Chinese EMs of S-mephenytoin share an unknown mutation making this enzyme incapable of demethylating diazepam while S-mephenytoin hydroxylation is unaffected.

Caution is, therefore, required for extrapolating the information obtained from one test probe to another, not only within one racial group but also between different racial groups. Therefore, one can conclude that each racial group should be examined separately for the evidence of polymorphic metabolism and the test probe most suitable for that group.

5. THE USE OF DRUG-DRUG INTERACTIONS TO LOWER DOSAGES

Sometimes drug-drug interactions are used to eliminate inter-individual differences in pharmacokinetics and make drug behaviour more predictable. Drug-drug interactions can also be used for economic purposes. Since its discovery and introduction into clinical practice, cyclosporin has become the most important immunosuppressive drug to prevent the rejection of transplanted organs. Cyclosporin, however, is expensive and the cost may preclude its use, especially when the transplant recipient is required to pay. As described above, cyclosporin is metabolised by CYP3A4, and CYP3A4 is very sensitive to interactions with other drugs and food constituents. Co-prescription of a second drug to lower the amount of cyclosporin needed (and the cost) has recently been introduced /88/. The drugs that are used most frequently for this purpose are fluconazole and cimetidine /88/. However, the financial benefits must be weighed against the risks of reduced compliance because of the adverse effects of the co-prescribed drugs. Poor or variable compliance will aggravate the natural interpatient variability and hence increase the risk of cyclosporin toxicity and/or organ rejection. It must be clear that with the limited availability of donor organs, such practices must be viewed with sceptism.

6. CONSEQUENCES OF GENETIC POLYMORPHISM FOR DRUG DEVELOPMENT

In the process of developing a new drug, there are several phases. In phase I studies (healthy subjects and patients) dose selection and

escalation studies are performed to find any effect of the drug as a function of the dose and the serum concentration. This will lead to pharmacokinetic and pharmacodynamic models. In phase II these models are refined and relevant information on interpatient variability of pharmacokinetics and pharmacodynamics is obtained, leading to identification of special populations that exhibit altered pharmacokinetics and pharmacodynamics. As illustrated above, the existence of poor metabolism and of inhibition or induction of CYPs by other drugs or chemicals may have important clinical implications because of the wide range of drugs that are metabolised by these enzymes. Because in phase I and II trials not all metabolic variants and drug-drug interactions can be studied, much effort is directed to the prediction of human *in vivo* enzymatic pathways from *in vitro* research, animal research and from computer models.

The availability of human liver banks with microsomes, human cytochrome P-450 enzymes and their mRNAs together with specific *in vitro* inhibitors of almost every human CYP enables extensive *in vitro* study of the metabolic pathways of new drugs. In addition, computer models are being developed to identify metabolic pathways at a very early stage of drug development /89/. Information on a new compound obtained preclinically should be used to optimise phase I and phase II clinical studies. The *in vitro* strategy allows for early recognition of the fact that a new drug will be subject to genetic polymorphism or to significant interactions during some stage of its metabolism. If the drug is judged to be of great therapeutic value, this should not necessarily lead to stopping further development. Alternatively, a "back-up" compound not exhibiting polymorphism or interactions may be preferred for further investigation.

However, there are limitations to the use of these techniques. One is the concentration that the drug reaches in the *in vivo* situation at the metabolising enzyme; *in vitro* the concentrations are chosen, but does this reflect the real *in vivo* concentration of the drug? A second limitation is that *in vivo* the enzyme system operates in concert with other enzyme systems, whereas *in vitro* isolated systems are studied. A third one is the lack of specific non-toxic *in vivo* markers for the enzymatic capacity of several enzymes. One example is imipramine, for which *in vitro* studies gave strong indications for a role of CYP1A2 and CYP3A4 in the demethylation process, which could not be confirmed *in vivo*, probably because of the lack of reliable *in vivo*

markers for CYP1A2 and CYP3A4. For most markers currently used to measure the oxidative enzyme activity for one drug, there is a lack of predictive value towards other drugs. Further investigation of the usefulness of other marker substances is needed. Another limitation is concerned with the extrapolation of data from animal studies to humans; although necessary, animal studies may be of limited value because of pronounced interspecies differences in enzyme constitution and kinetics. However, with the availability of these techniques, disasters, for example the observed cardiotoxicity of terfenadine and cisapride when these drugs are combined with inhibitors of CYP3A4, can hopefully be avoided in the future.

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