Pharmacogenomics in and its Influence on Pharmacokinetics

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Abstract

CYP1A2 is involved to a major extent in the metabolism of several drugs (imipramine, clozapine, fluvoxamine, olanzapine, theophylline, acetaminophen, propranolol, and tacrine) as well as of diet components (methylxanthines), endogenous substrates (estrogens), numerous aryl, aromatic and heterocyclic amines, and polycyclic aromatic hydrocarbons. It is inducible, notably by cigarette smoking, diet habits

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F. J. Hock, M. R. Gralinski (eds.), *Drug Discovery and Evaluation: Methods in Clinical Pharmacology*,
https://doi.org/10.1007/978-3-319-56637-5_25-1

such as consumption of cruciferous vegetables (e.g., broccoli, watercress, collard greens, Brussels sprouts, and mustard) and of charbroiled meats, some drugs (omeprazole, phenytoin, and rifampicin) and is a target enzyme for the development of some cancers. Up to now, more than 25 CYP1A2 alleles have been detected. Probe drugs for CYP1A2 phenotyping are caffeine and theophylline. For safety concerns and drug availability, the preferred probe is caffeine. Caffeine 3-demethylation is mediated by CYP1A2, and accounts for 80% of caffeine clearance. Caffeine is also a probe drug for N-acetyltransferase and xanthine oxidase (Clin Pharmacol Ther 53:203–514, 1993).

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The understanding of the role of pharmacogenetics in drug metabolism expanded greatly in the 1990s. This is mainly due to technological improvements in gene scanning and gene variant identification. The number of variant alleles identified for genes coding for drug metabolizing enzymes (DME) considerably increased in the early 2000s, and continues to increase. The clinical consequences – or at least genotyping–phenotyping relationships – of DME polymorphisms have not been demonstrated for all variants. In the text below, only those DME allele variants will be mentioned for which significant changes in enzyme activity have been found using probe drugs. Comprehensive information on the nomenclature of cytochrome P450 (CYP) alleles can be found at www.imm.ki.se/CYPalleles and Phase I and Phase II DMEs at www.pharmgkb.org/index.jsp.

Phase I Enzymes

CYP1A2

Purpose and Rationale

CYP1A2 is involved to a major extent in the metabolism of several drugs (imipramine, clozapine, fluvoxamine, olanzapine, theophylline, acetaminophen, propranolol, and tacrine) as well as of diet components (methylxanthines), endogenous substrates (estrogens), numerous aryl, aromatic and heterocyclic amines, and polycyclic aromatic hydrocarbons. It is inducible, notably by cigarette smoking, diet habits such as consumption of cruciferous vegetables (e.g., broccoli, watercress, collard greens, Brussels sprouts, and mustard) and of charbroiled meats, some drugs (omeprazole, phenytoin, and rifampicin) and is a target enzyme for the development of some cancers. Up to now, more than 25 CYP1A2 alleles have been detected. Probe drugs for CYP1A2 phenotyping are caffeine and theophylline. For safety concerns and drug availability, the preferred probe is caffeine. Caffeine 3-demethylation is mediated by CYP1A2, and accounts for 80% of caffeine clearance. Caffeine is also a probe drug for N-acetyltransferase and xanthine oxidase (Kalow and Tang 1993).

Procedure

Phenotyping: A fixed or weight-adjusted dose of caffeine (solution, tablet, and coffee) ranging from 1 to 3 mg/kg is administered. Diet requirements have to be respected (stable xanthine-free diet avoiding beverages such as coffee, tea, cola, chocolate, no food component with CYP1A2-

inducing properties) during the test period. As smoking is known to induce CYP1A2, control of stable smoking status is mandatory.

There are two commonly used and robust methods for phenotyping. The first one measures caffeine (1,3,7-methylxanthine) and its N-demethylated metabolite 1,7-dimethylxanthine (paraxanthine) in a plasma or saliva sample collected within 5–7 h post-caffeine dosing (Fuhr and Rost 1994). The second one uses the assay of the metabolites 1-methylurate (1 U), 1-methylxanthine (1X), 5-acetylamino-6-formylamino-3-methyluracil (AFMU), and 1,7-dimethylurate (17 U) levels in urine collected at least for 8 h post-dosing (Campbell et al. 1987; Rostami-Hodjegan et al. 1996).

Commonly used methods for caffeine and metabolite(s) assay in plasma or urine involve an extraction step followed by HPLC with UV detection (Krul and Hageman 1998a; Rasmussen and Bosen 1996; Schreiber-Deturmeny and Bruguerolle 1996). Urine needs to be acidified (pH 3.0–3.5) before sample freezing.

Genotyping: Reduced activity has been reported for CYP1A2*1C and CYP1A2*1F alleles in smoking subjects. Induction of CYP1A2 activity has been associated with these alleles, but the effect of CYP1A2*1F mutation on CYP1A2 activity has not been confirmed (Nordmark et al. 2002). In Caucasians, frequency of the CYP1A2*1C and CYP1A2*1F variants is about 1% and 33%, respectively (Sachse et al. 2003).

Evaluation

Metabolic ratios (MR) used are plasma 17X/137X and urinary (1 U + 1X + AFMU)/17 U.

In controlled conditions, in nonsmoking young and elderly subjects, intraindividual and interindividual variability in 17X/137X MR was about 17% and 47%, respectively, with no effect of age (Simon et al. 2003). A 70-fold range in MR has been observed in smoking and non-smoking female Caucasian subjects using the urinary MR (Nordmark et al. 1999). Up to 200-fold differences were found using the urinary test. Lower variability is expected using the plasma caffeine test.

Higher CYP1A2 activity in men versus women has been reported, though inconsistently, and in children. Higher MR is usually observed in smokers versus nonsmokers, when population sample size is large. Pregnancy and oral contraceptives intake were found to decrease CYP1A2 activity (Abernathy and Todd 1985; Caubet et al. 2004; Kalow and Tang 1993). CYP1A2 activity was found lower in colorectal patients versus controls (Sachse et al. 2003).

Large variability in CYP1A2 activity explains that its distribution has been described unimodal, bimodal, or trimodal. Poor metabolizers (PM, characterized with a MR <0.12) have been identified in Chinese population and represented about 5% of the population tested, whereas PM could represent 5–10% of Caucasian populations and 14% in Japanese population (Ou-Yang et al. 2000).

Critical Assessment of the Method

Numerous studies have shown good correlation between the 17X/137X plasma MR and caffeine systemic clearance, and plasma MR is considered more robust than the urinary one, since this last one can be affected by the effect of urinary flow on metabolite renal clearances.

Currently, no relationship between CYP1A2 genotype characteristics and CYP1A2 activity, as assessed by the caffeine test, has been usually found. Some associations have been found in specific genetic and environmental conditions (Han et al. 2001). Non-well-controlled conditions for urine sample collection, the effects (induction) linked to environmental factors may overcome the role of CYP1A2 polymorphism, which can explain the paucity of clear associations between CYP1A2 genotyping and phenotyping.

Further investigations are needed to characterize the effect of variants (SNPs, haplotypes) on CYP1A2 activity.

Modifications of the Method

Recent drug assay development involved LC-MS methods (Caubet et al. 2004; Kanazawa et al. 2000). A less practical breath test, using ^{13}C or ^{14}C labeled caffeine, can also be used (Kalow and Tang 1991).

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CYP2C9

Purpose and Rationale

CYP2C9 is involved in the hydroxylation of about 16% of drugs (Schwarz 2003), including drugs with narrow therapeutic index such as anticoagulants (warfarin, acenocoumarol, and phenprocoumon active S-enantiomers), and anticonvulsivants (phenytoin and hexobarbital), as well as numerous antidiabetic agents (i.e., tolbutamide, glibenclamide, and glipizide), antihypertensive drugs (losartan, irbesartan), nonsteroidal anti-inflammatory agents (i.e., diclofenac, ibuprofen, and celecoxib), diuretic (torsemide), and antirheumatoid agents (leflunomide).

A couple of CYP2C9 variants – mainly CYP2C9*2 and CYP2C9*3 – code for in vivo decreased activity, and two – CYP2C9*6 and CYP2C9*15 – have been reported to be associated with no activity. In Caucasian populations, CYP2C9*2 and CYP2C9*3 are encountered in 20–25% of subjects, while these genotypes have been found in less than 5% of East Asian subjects (Rosemary and Adithan 2007).

Probe drugs regularly used for CYP2C9 phenotyping are tolbutamide, warfarin, phenytoin, and losartan. Diclofenac, flurbiprofen, phenprocoumon, and torsemide have also been used. For safety concerns, the current preferred probe is tolbutamide, despite some risk of hypoglycemia.

Procedure

Phenotyping: The method measures tolbutamide, its CYP2C9-formed 4′-hydroxylated metabolite hydroxytolbutamide and the subsequent carboxytolbutamide metabolite, the latter formed by dehydrogenase enzymes. The urinary excretion of these two metabolites represented more than 85% dose of administered tolbutamide (Veronese et al. 1990, 1993).

Subjects receive a single oral 500 mg tolbutamide tablet in usual Phase I standard controlled conditions, with care to be paid to blood glucose. Urine is collected from drug intake to 8 or 24 h post-dosing.

The assay of tolbutamide and its metabolites is usually performed using HPLC and UV or fluorescence detection (Csillag et al. 1989; Veronese et al. 1990; Kirchheiner et al. 2002a, b; Hansen and Brosen 1999).

Genotyping: About two-third of Caucasian subjects express the wild genotype C9*1/*1. C9*1/*2 and C9*1/*3 heterozygote variants are expressed in 15–25% and 7–16% of Caucasian subjects, whereas the frequency of other variants is lower: 0.5–2.5%, 1–3%, and <1–1.5% for C9*2/*2, C9*2/*3, and C9*3/*3 variants, respectively (Scordo et al. 2001; Lee et al. 2002a, b; Schwarz 2003). More than 95% of Afro-American subjects express the wild genotype C9*1/*1 (Lee et al. 2002a, b). In Asian populations, CYP2C9*1/*3 is expressed in 2–8% subjects, but CYP2C9*2 is absent or extremely rare (Rosemary and Adithan 2007; Schwarz 2003; Xie et al. 2002). Overall, it has been estimated that 0.2–1% and 2–3% of Caucasian and Asian population could be qualified as PM, respectively (Meyer 2000).

Evaluation

The urinary MR (MR, hydroxytolbutamide + carboxytolbutamide)/tolbutamide is generally used. There is a large interindividual variability in MRs in subjects with the same genotype. Different studies performed with different probe drugs (Yasar et al. 2002a, b; Kirchheiner et al. 2002a, b, 2003a; Lee et al. 2002a, b; Miners and Birkett 1998; Morin et al. 2004), highlighted that a PM status could be given to subjects who are homozygous for CYP2C9*3, or expressing CYP2C9*2/*3 variant, but intermediate situations – from extensive to slow metabolizer status – may vary not only among different allele combinations but also with the probe drug used.

Oral contraceptives were found to inhibit CYP2C9 activity using losartan for phenotyping (Sandberg et al. 2004).

Critical Assessment of the Method

The tolbutamide test has the most convincing ability to discriminate between genotype variants and pharmacokinetics. There could be an analytical issue linked to the urine assay precision, as the urinary concentrations of the parent drug are very low in comparison with those of its metabolites.

To date, the CYP2C9*3 variant has been the only one found influencing significantly drug pharmacodynamics for warfarin, acenocoumarol (Sandberg 2003; Morin et al. 2004; Versuyft et al. 2003), glipizide, and glyburide (Kirchheiner et al. 2002a, b) or drug side effects (Sevilla-Mantilla et al. 2004). Inconstant results were found regarding tolbutamide effects (Kirchheiner et al. 2002a, b; Shong et al. 2002). For anticoagulants, the possession of CYP2C9*2 and CYP2C9*3 variants was associated with decreased warfarin dose requirement in patients, and an increased risk of adverse events such as bleeding (Daly and King 2003). An Afro-American subject with only the CYP2C9*6 variant exhibited serious phenytoin side effects associated with a marked impaired elimination of the drug (Kidd et al. 2001).

The variability of CYP2C9 activity observed among ethnic groups cannot be explained with our current knowledge on CYP2C9 variant alleles distribution (Xie et al. 2002).

Modifications of the Method

Losartan (25 mg dose) has been proposed as a safer alternative to tolbutamide. The determination of losartan/E3174 (oxidized metabolite) ratio in 0–8 h urine or in plasma at 6 h post-dosing have been proposed (Yasar et al. 2002a, b; Sekino et al. 2003). However, in a comparative study in 16 subjects, a better correlation between genotyping and phenotyping was found with tolbutamide, as compared to losartan or flurbiprofen, though there was no subject with the C9*2/*3 or C9*3/*3 variants (Lee et al. 2003).

Recently, a 125 mg tolbutamide dose has been validated, with proposal of the use of just one blood sample collected 24 h post-dosing. Its safer use needs the drug to be assayed using LC-MS/MS methodology (Jetter et al. 2004).

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CYP2C19

Purpose and Rationale

CYP2C19 contributes to the metabolism of about 8% of drugs (Rogers et al. 2002), including S-mephenytoin, proton pump inhibitors (omeprazole, lansoprazole, and pantoprazole), tricyclic antidepressants (amitriptyline, imipramine, clomipramine, and citalopram), benzodiazepines (diazepam and flunitrazepam), torsemide, fluvastatin, and proguanil. Two main variants – CYP2C19*2 and CYP2C19*3 – are coding for in vivo nil activity, as well as CYP2C19*4, *5, *6, *7, and *8 variants. About 15–20% Asians, 4–7% Black Africans, and 3% Caucasians are PM (Scordo et al. 2004).

Probe drugs used for CYP2C19 phenotyping are mephenytoin, omeprazole, and proguanil. The most currently used probe drug is omeprazole.

Procedure

Phenotyping: The method measures omeprazole, and its CYP2C19-formed 5-hydroxylated metabolite in plasma.

Subjects receive a single oral 20 or 40 mg omeprazole capsule in usual Phase I standard controlled conditions. Plasma can be collected from drug intake up to 24 h post-dosing, or only one plasma sample is collected at 2 or 3 h post-dosing.

The assay of omeprazole and its metabolite is usually performed using HPLC and UV detection (Lagerstrom and Persson 1984; Ieri 1996; Yim et al. 2001; Tybring et al. 1997) or LC-MS/MS assay (Kanazawa et al. 2002).

Genotyping: The two alleles CYP2C19*2 and CYP2C19*3 account for quite all PM in Asians (>99%) and Black Africans, but defective alleles have not been fully characterized in 10–15% Caucasians. The CYP2C19*2 allele is the most frequent in Asian populations (30% in Chinese), as well as in Black Africans (about 17%) and in Caucasians (about 15%) (Xie et al. 2001). The CYP2C19*3 accounts for about 25% of inactive forms in Orientals, and is extremely rare in Caucasians (Scordo et al. 2004; Rosemary and Adithan 2007).

Evaluation

The AUC or plasma ratio of omeprazole to 5-hydroxyomeprazole is used.

As expected, homozygous PM subjects have lower metabolic activity as compared to heterozygous PM subjects, and potential interethnic difference has been noticed within a genotype (Yin et al. 2004).

Decreased CYP2C19 activity has been observed with oral contraceptives containing ethinylestradiol (Tamminga et al. 1999; Laine et al. 2000).

Critical Assessment of the Method

Omeprazole hydroxylation rate correlates with S-mephenytoin hydroxylation rate, which was initially the CYP2C19 probe drug (Andersson et al. 1990; Chang et al. 1995; Balian et al. 1995). The alternate pathway – conversion of omeprazole to its sulfone derivative – that is mediated via CYP3A4, does not influence the CYP2C19 pathway of omeprazole (Balian et al. 1995).

Time-dependent kinetics of omeprazole limits its use for phenotyping during chronic therapy (Gafni et al. 2001). CYP2C19 phenotyping with omeprazole may be affected by age, liver disease, and omeprazole therapy (Kimura et al. 1999).

Interethnic differences observed with different CYP2C19 substrates for subjects with same genotype have been attributed to differences in substrate specificity or enzyme isoforms (Bertilsson et al. 1992). The clearance of omeprazole is higher in Caucasian extensive metabolizers (EM) than in Oriental EM, due to a higher proportion of heterozygous EM in this latter population (Ishizaki et al. 1994).

Modifications of the Method

It has been proposed to use omeprazole for both CYP2C19 and CYP3A4 phenotyping (Gonzalez et al. 2003).

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CYP2D6

Purpose and Rationale

CYP2D6 is involved significantly in the metabolism of drugs mainly used in CNS (antidepressants, i.e., imipramine, paroxetine, citalopram; neuroleptics, i.e., haloperidol, risperidone), or cardiovascular (β-adrenoceptor blockers, i.e., metoprolol; antiarrhythmics, i.e., propafenone and flecainide) disorders. Significant interethnic and interindividual intraethnic differences in CYP2D6 activity have been found. It is found that 5–10% Caucasians, 6–8% Afro-Americans, and only 1% Asians have reduced CYP2D6 activity, and exhibit the PM phenotype. Expression of CYP2D6 has been shown to be polymorphic with up to now more than 80 genetic variants detected for the encoding gene, with more than 15 encoding for inactive enzyme. Probe drugs for CYP2D6 phenotyping are dextromethorphan, debrisoquin, sparteine, and metoprolol. For safety concerns and drug availability, the preferred probe is dextromethorphan (DM) (Schmid et al. 1985).

Procedure

Phenotyping: The method measures DM and its O-demethylated metabolite, dextrorphan (DX), which is formed by CYP2D6. DM and DX, and other metabolites, are excreted in urine, mainly as glucuronide conjugates.

Subjects receive a single oral 10–30 mg DM (generally hydrobromide salt syrup) dose. Urine is collected from drug intake to 8 h post-dosing. Other collection times (0–6, 0–10, 0–12, or 0–24 h) can be used, but short collection intervals might lead to increased intra-subject variability.

Urine is first hydrolyzed with β-glucuronidase. Then, different methods can be used involving DM and DX extraction, followed either by HPLC and fluorescence detection (Chladek et al.

1999; Hoskins et al. 1997) or capillary gas chromatography (Wu et al. 2003).

Genotyping: The incidence of alleles coding for inactive enzymes varies between populations: three "population specific" alleles are CYP2D6*4 in Caucasians, *10 in Asians, and *17 in Africans (Bertilsson et al. 2002). CYP2D6*3, *4, *5, *6 are the main inactive alleles producing the PM phenotype in Caucasians, with CYP2D6*4 most commonly associated with the PM phenotype. By far, the most frequent null allele – not encoding a functional protein product – is CYP2D6*4 with a frequency of 20–25% in Caucasians (Zanger et al. 2004). The frequency of the *17 allele – associated with decreased enzyme activity – is high in Black Africans and in Black Americans, but practically absent in Caucasian populations (Bapiro et al. 2002; Gaedigk et al. 2002; Zanger et al. 2003). Four potential subgroups – ultrarapid metabolizers (UM), extensive metabolizers (EM), intermediate metabolizers (IM), and poor metabolizers (PM) – have been defined based on the genotype–phenotype relationships.

In Caucasian subjects, it has been recommended for "routine test" to genotype for alleles *1, *3, *4, *5, *6 that allow to detect 86–100% of PM (Sachse et al. 1997). To assign correct phenotype in nearly 100% subjects, *9 and *10 variants should also be determined.

Evaluation

Subjects with a DM/DX MR >0.3 are PM. Subjects with DM/DX <0.03 are EM. Those with $0.03 < \mathrm{MR} < 0.3$ are IM.

No difference or slightly higher CYP2D6 activity in females has been found when comparing to male subjects (Hägg et al. 2001; McCune et al. 2001).

Relationship between phenotyping and genotyping is investigated by plotting log MR versus CYP2D6 allele combinations (Chou et al. 2003).

Critical Assessment of the Method

The method is widely used due to easy and safe administration. High intrasubject variability limits the test for discriminating between EM and UMs (Zanger et al. 2004).

The method is not appropriate in patients with renal impairment, due to reduced renal excretion of DM glucuronide metabolites. Sparteine has been recommended as a probe for this population and to discriminate between the four phenotypes UM, EM, IM, and PM. The DM/DX MR does not allow for consistent differentiation between CYP2D6 EM with one or two active alleles.

Modifications of the Method

Assays have been developed to determine DM and DX in plasma or saliva (Bolden et al. 2002; Hu et al. 1998; Chladek et al. 2000; Härtter et al. 1996). The use of saliva or plasma for CYP2D6 phenotyping has been developed for subject convenience, or for the development of single point methods to be easily incorporated in the "cocktail methods." Good correlation between MRs calculated from plasma, saliva samples and those obtained from urine has been observed.

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CYP3A

Purpose and Rationale

CYP3A is the predominant P450 subfamily (CYP3A4, CYP3A5, CYP3A7, and CYP3A43) in the human liver, and contributes significantly to the metabolism of many (at least 50%) drugs in numerous therapeutic classes. CYP3A4 is the major CYP present notably and predominantly in the liver and the small intestine, and interindividual variability in the level of its expression is very high – 20-fold or more (Shimada et al. 1994). CYP3A5 shares rather similar tissue distribution with CYP3A4, but is preferentially expressed in the lung. It represents generally a few percentage of total CYP3A as compared to CYP3A4 (exceptions are esophagus and prostate, specific for CYP3A5, and kidney in which CYP3A5 is predominantly expressed). CYP3A4 and CYP3A5 exhibit overlapping substrate specificity, and there is currently no specific CYP3A5 probe drug. CYP3A7 is primarily the major fetal CYP3A enzyme.

Most of drugs biotransformed with CYP3A are also P-glycoprotein substrates (noticeable exceptions are midazolam and nifedipine). CYP3A and P-glycoprotein contribute substantially to the first-pass elimination of highly cleared CYP3A substrates when orally administered. However, CYP3A4 and P-glycoprotein activities are not coordinately regulated in the liver and in the intestine (von Richter et al. 2004).

Currently, 40 and 24 alleles have been identified for CYP3A4 and CYP3A5, respectively. Expression of CYP3A5 varies greatly among individuals (Lamba et al. 2002).

Due to multiple confounding factors, such as those involved in endogenous expression of CYP3A regulatory factors, numerous exogenous factors (environment, diet), the interplay between CYP3A and transporters in regulating drug disposition, the establishment of consistent relationships between CYP3A genotype and phenotype is actually a challenge (Wilkinson 2004). Currently, the value of CYP3A genotyping in drug development is far from being clinically useful.

The most used and validated probe drugs for CYP3A phenotyping are midazolam and ^{14}C–erythromycin (Watkins 1994). Alfentanyl, alprazolam, dapsone, DM, lidocaine, nifedipine, omeprazole, quinine, and verapamil have also been used but less frequently, and CYP3A specificity for some of them has been questioned. The "endogenous" 6β-hydroxycortisol test (measurement of 6β-hydroxycortisol: cortisol ratio in urine) is only useful for detecting CYP3A induction, and may be influenced by renal CYP3A activity.

Due to intraindividual differences in the liver and the intestinal CYP3A activity, phenotyping test results are related to the probe drug route of administration.

Procedure

Phenotyping

Midazolam test: Midazolam is primarily metabolized to 1′-hydroxymidazolam by CYP3A. It is rapidly and completely absorbed after oral administration (Gorski et al. 1998). It is the probe of choice to assess intestinal and hepatic or hepatic CYP3A activities only, after oral (Thummel et al. 1996) or intravenous administration, respectively.

Oral test doses are 2, 5, or 7.5 mg (as a solution). IV doses are 0.015, 0.025, or 0.05 mg/kg, or 1 or 2 mg per subject, as a 2–30-min infusion.

Blood samples are collected over a 6-h period. Numerous GC, GC/MS, HPLC/UV, or LC/MS methods have been developed for plasma midazolam assay (Lepper et al. 2004; Frison et al. 2001).

^{14}C-erythromycin breath test or ERMBT: CYP3A4 catalyzes the N-desmethylation of [^{14}C N-methyl] erythromycin. The test consists of the measurement of a single breath expired $^{14}CO_2$ collection obtained at 20 min following the IV administration of a 0.03 mg dose of ^{14}C–erythromycin (2–4 μCi administered) (Watkins 1994). This test is used for assessing hepatic CYP3A activity.

Genotyping: Allelic CYP3A4 gene variants are rare. No impact of the presence of the most common CYP3A4*1B mutation (with a frequency ranging from 0% in Chinese and Japanese to 45% in Afro-Americans) on midazolam, erythromycin, or nifedipine clearance has been evidenced. Most significant mutations are observed for CYP3A5 and CYP3A7. Further information on polymorphic expression of CYP3A5 and CYP3A7 can be found in the review by Lamba et al. (2002).

Evaluation

A complete pharmacokinetic profile is required to assess midazolam clearance, and is therefore more invasive than the ERMBT; however, the latter requires specific logistics for radiolabeled material use. The midazolam or ERMBT phenotype tests are used for dose individualizing of narrow therapeutic index CYP3A-metabolized drugs such as anticancer agents. The ratio 1-′-hydroxymidazolam/midazolam has generally been found not useful for phenotyping.

Within a population of similar demographic and health characteristics, a four- to sixfold range in the metabolic clearance of a CYP3A-drug substrate is usual, with common individual outliers exhibiting high or low activity (Lamba et al. 2002).

Critical Assessment of the Method

Midazolam clearance has been found to correlate with hepatic CYP3A levels (Thummel et al. 1994) as well as ERMBT results (Lown et al. 1992). However weak, inconstant, or lack of correlations between midazolam and ERMBT test results have been observed, which could be explained by binding to different CYP3A active sites. In addition, contrary to the midazolam test, the ERMBT does not capture CYP3A5 activity.

An ethnic difference – that could be drug-specific – in CYP3A4 activity has been observed for few CYP3A4 substrates (alprazolam and nifedipine), with a lower clearance in Asians than in Caucasians (Xie et al. 2001).

CYP3A4 and CYP3A5 genotyping tests could not explain sufficiently the interindividual variability observed in midazolam pharmacokinetics (Eap et al. 2004a).

Modifications of the Method

The combined use of IV midazolam and oral ^{15}N-midazolam or of the ERMBT and oral midazolam tests have been proposed to assess simultaneously the contributions of liver and intestine in CYP3A activity (Gorski et al. 1998; McCrea et al. 1999). The administration of orally given midazolam followed by an intravenous administration has also been validated (Lee et al. 2002c). A low oral 75 μg oral dose has recently been proposed, but needs large-scale validation (Eap et al. 2004b).

Modifications of the ERMBT have been described to improve its predictability in drug clearance estimations in cancer patients (Rivory et al. 2000).

A single blood sample for midazolam assay at 4 h post-dose has been reported as good estimator for IV or oral midazolam clearance determination (Lin et al. 2001).

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Other CYPS

This section summarizes succinctly the current knowledge on some other CYPs, its role in drug metabolism and its genetic impact have been more recently investigated as compared to other CYPs.

CYP2A6

CYP2A6, primarily expressed in the liver, is the major CYP (the sole at usual low concentrations) involved in nicotine oxidation, and is also involved in the metabolism of carcinogen or procarcinogen compounds (such as nitrosamines and aflatoxins). A couple of drugs is metabolized by CYP2A6: chlormethiazole, coumarin, disulfiram, halothane, valproic acid, and others (Oscarson 2001). CYP2A6 PM is less than 1% in Caucasians but up to 20% in Orientals (Oscarson 2001; Raunio et al. 2001; Xu et al. 2002). The most "in vivo deficient" alleles for PM status are CYP2A6*2 and CYP2A6*4, rather common in Orientals (15% in Chinese, 20% in Japanese). The important role of CYP2A6 in nicotine metabolism was shown in an epidemiological study, revealing that the CYP2A6 genotype was a major determinant for smoking behavior and susceptibility to tobacco-related lung cancer (Fujieda et al. 2004).

Phenotyping has been performed in some countries with coumarin (not available in all countries), despite some limitations with data accuracy obtained with the analytical methods used (Pelkonen et al. 2000; Cok et al. 2001). The test assesses the amount of 7-hydroxycoumarine (free and conjugated) in urine after ingestion of 2–5 mg coumarine by the subjects. Nicotine has also been used as the probe drug for CYP2A6 in vivo activity testing. Recent investigations using pilocarpine as probe demonstrated that PM status was associated with two inactive CYP2A6 alleles, CYP2A6*4A, CYP2A6*7, CYP2A6*9, or CYP2A6*10 (Endo et al. 2008).

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CYP2B6

CYP2B6 has been estimated to represent 1–10% of the total hepatic CYP content. It catalyzes bupropion hydroxylation, S-mephenytoin N-demethylation, and is involved in the metabolism of cyclophosphamide, ifosfamide, mianserin, efavirenz, artemisinin, and propofol (Turpeinen et al. 2006). CYP2B6*6 has been associated with reduced bupropion clearance in vitro (Hesse et al. 2004), but not in vivo whereas a moderate clearance increase was observed with CYP2B6*4 (Kirchheiner et al. 2003b). Multiple gene polymorphisms have resulted in phenotypic null alleles (Lang et al. 2004). Pharmacokinetics of the anti-HIV drug efavirenz has been associated with CYP2B6–G516 T polymorphism (Saitoh et al. 2007).

Bupropion (150 mg dose) has been proposed for phenotyping, but it is recommended to administer body weight-adjusted doses (Faucette et al. 2000). Efavirenz may also be a valuable probe for CYP2B6 (Ward et al. 2003).

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CYP2C8

CYP2C8 is involved in the metabolism of arachidonic acid, all-trans retinoic acid, paclitaxel, amiodarone, amodiaquine, repaglinide, rosiglitazone, torsemide, troglitazone, and zopiclone. Most of these drugs are also metabolized by CYP3A4. Recently, the potential contribution of CYP2C8 to the metabolism of NSAIDs in addition to the well-known CYP2C9 role has been highlighted for ibuprofen (Garcia-Martin et al. 2004). The CYP2C8*3 allele (present in 13% and 2% of Caucasians and Afro-American subjects, respectively) has been shown in vitro deficient for paclitaxel and arachidonic acid metabolism (Dai et al. 2001; Bahadur et al. 2002). For the antidiabetic repaglinide, unexpected in vivo lower exposure was observed in subjects with CYP2C8*1/*3 genotype, without any pharmacological consequences (Niemi et al. 2003). For ibuprofen, reduced clearance of the R (−) enantiomer was related to CYP2C8*3 allele, and reduced clearance of the S(+) enantiomer was influenced by CYP2C8*3 and CYP2C9*3 alleles. In subjects homozygous or double heterozygous for these variants (8% of 130 subjects evaluated), the clearances of ibuprofen were only 7–27% of the clearances observed in subjects with no CYP mutations. A strong association between CYP2C8*3 and CYP2C9*2 occurrence has been characterized in a large Swedish population, highlighting linkage between CYP2C8 and CYP2C9 polymorphisms (Yasar et al. 2002c).

Further in vitro/in vivo investigations are needed to assess the relationship between CYP2C8 (and CYP2C9) polymorphisms and drug metabolic clearance, in order to address the clinical relevance of CYP2C8 genotyping.

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CYP2E1

CYP2E1, an ethanol-inducible CYP, activates some procarcinogens such as nitrosamines, is involved in the metabolism of endogenous substrates (steroids and bile acids), alcohols, xanthines, volatile chemicals (toluene, benzene, and halocarbons), but of few drugs (chlorzoxazone, etoposide, dapsone, and high-dose acetaminophen) (Lieber 1997). Seven alleles, 13 genetic mutations have been described, but no genotyping–phenotyping relationships have been well established to date. Based on safe use and CYP selectivity (though CYP1A1, CYP1A2 have been found involved in its biotransformation in vitro), chlorzoxazone is the only in vivo probe drug to phenotype CYP2E1 activity, toward assessment of its 6-hydroxylation (Ono et al. 1995; Lucas et al. 1999; Ernstgard et al. 2004). Due to dose-dependent metabolism, the dose should be preferably administered on a mg/kg basis (10 mg/kg rather than the common 250 or 500 mg doses). Relatively low intraindividual variability in chlorzoxazone metabolism has been observed. Measurement can be done in urine or in plasma, after enzymatic hydrolysis of 6-chlorzoxazone glucuronide, using HPLC and UV detection or LC/MS/MS methods (Frye and Stiff 1996; Frye et al. 1998; Scoot et al. 1999). The use of plasma metabolite ratio determined with only one plasma sample – at 2 h post-dosing – has been recently validated.

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Phase II Enzymes

With the exception of N-acetyltransferases (detailed below), there are few deficiencies in Phase II drug metabolism enzymes that have resulted in clinically significant effects. Each Phase II enzyme class is most often a superfamily of enzymes, and usually there is large interindividual and interethnic variability in drug conjugations, and overlapping substrate specificity exists for numerous isoenzymes. Despite the crucial role of conjugation enzymes in xenobiotic metabolism, the functional significance of enzyme polymorphism is only known for few substrates. Therefore, with the exception of the caffeine and thiopurine methyltransferase (TPMT) tests (see below), no probe test drug has been yet investigated for in vivo phenotyping and validated to assess phenotyping–genotyping relationships. Nevertheless, some important aspects of enzyme polymorphism on the pharmacokinetics of drugs with narrow therapeutic index are summarized below.

N-Acetyltransferases

Purpose and Rationale

N-acetyltransferases type I (NAT1) and type II (NAT2) catalyze N- and O-acetylation reactions involved in the metabolism of drugs containing arylamino, hydroxyl, sulfhydryl groups and hydrazine structure, and also in environmental carcinogens (such as those present in tobacco smoke, or in diet such as charcoal-broiled food) (Weber and Hein 1985). Pending on the drug, and on the interplay between CYPs and N-acetylases (and other Phase II conjugation enzymes) in xenobiotic metabolism, the impact of subject status "poor acetylator" or "rapid acetylator" on drug activity and/or toxicity may vary, and then is drug specific. NAT1 and NAT2 exhibit a high degree (81%) of amino-acid sequence homology, and share common substrates (Meisel 2002) but coding genes loci are regulated independently. Main NAT2 drug substrates are isoniazid, sulfonamides, procainamide, hydralazine, acebutolol, aminoglutethimide, and dapsone.

Para-aminosalicylic and para-aminobenzoic acids are considered specific substrates for human NAT1, and sulfamethazine, isoniazid, procainamide, and dapsone are considered specific substrates for human NAT2 (Butcher et al. 2002). NAT1 is considered as ubiquitously distributed in the body, whereas NAT2 is expressed in liver and intestinal mucosa.

Polymorphic N-acetylation was first described for isoniazid in the 1950s and is the first example of interindividual pharmacogenetic variability.

Until 2007, about 30 and more than 50 variant alleles have been described for NAT1 and NAT2, respectively. At http://N-acetyltransferasenomenclature.louisville.edu overviews on the NAT alleles can be found. The presence of some NAT1 variants, as well as NAT2 variants, has been linked to increased susceptibility to some cancers (notably bladder and colon cancers), and NAT2 polymorphism associated with some drug-induced diseases such as lupus erythematosus (hydralazine and procainamide), Stevens–Johnson or Lyell syndromes (sulfonamides).

Significant interethnic and geographic differences in NAT2 activity have been found. Slow acetylators represent 40–70% Caucasians and 10–20% Asians. High acetylation capacity has been reported in 5% Caucasians (Meyer and Zanger 1997).

Probe drugs for NAT1 phenotyping is PAS, and for NAT2 phenotyping are caffeine, sulfamethazine, procainamide, isoniazid, and dapsone. In vivo testing for NAT2 has been proved useful for drug monitoring to avoid potential side effects generally observed in slow metabolizers (the exception was the anticancer agent amonafide, with myelotoxicity observed in rapid acetylators). The most used test to identify rapid and slow acetylators is the caffeine test, which is described thereafter, though the N-acetylation step takes place after the N-desmethylation of caffeine by CYP1A2 followed by the biotransformation into an unstable intermediate.

Procedure

Phenotyping: Caffeine is metabolized by CYP1A2, NAT2, and xanthine oxidases. The methods could involve the measurement of 5-acetyl-formylamino-3-methyluracil (AFMU), 5-acetyl-amino-3-methyluracil (AAMU, degradation product of AFMU), 1-methyl-xanthine (1MX), and 1-methyluric acid (1MU) in 0–8, 0–12, 0–24 h urine of subjects orally given 200 mg or 2–3 mg/kg caffeine after a xanthine-free regimen. The common MR used is AFMU/1MX, but the AFMU/(AFMU +1MX + 1MU) is more discriminating (Relling et al. 1992; Rostami 1995) and should be used when xanthine-oxidase inhibitors may be present (Fuchs et al. 1999). Other ratios such as AFMU/(1MX + 1MU), or AAMU/1MX, AAMU/(AAMU +1MX + 1MU) have been validated (Tang et al. 1991; Nyeki et al. 2002).

The most common methods to assay caffeine and its metabolite in urine used HPLC with UV detection (Grant et al. 1984; Krul and Hageman 1998b) or mass spectrometry (Baud-Camus et al. 2001).

Genotyping: Mutations of NAT2*5, NAT2*6, NAT2*7, NAT2*14, and NAT2*17 alleles are associated with a slow acetylation phenotype for homozygous subjects (Butcher et al. 2002).

There are large differences among ethnic groups regarding alleles' frequency. High frequency (>28%) of NAT2*5 alleles has been observed in Caucasians and Africans, and of NAT2*7 in Asians (>10%) and of NAT2*14 in Africans (>8%), this last one being <1% in Caucasians and Asians (Meyer and Zanger 1997).

Evaluation

Caffeine test: Subjects with a AFMU/1MX ratio < 0.55 or a AFMU/(AFMU +1MX + 1MU) ratio < 0.26 are slow acetylators (Fuchs et al. 1999). Higher activity has been observed in black as compared to white subjects (Relling et al. 1992), and a gender effect has generally not been observed (Kashuba et al. 1998).

Critical Assessment of the Method

Depending on the probe drug used and on the experimental method, 2 or 3 acetylator types can be described: slow, intermediate, and rapid; the intermediate one being not always distinguished from the rapid one. Phenotype distribution has been considered as a continuous variable (Meisel 2002). Due to slow postnatal maturation of the acetylation enzymatic systems, the acetylation status is evolving in newborns and infants, and depends on the probe drug used (Rane 1999).

Good relationships between genotyping and phenotyping tests have been reported (Meisel et al. 1997; Kita et al. 2001).

The urinary caffeine test is not based on assays of specific substrates and products of NAT2 ("including" other metabolism pathways involving at least xanthine-oxidases), and is affected by diet habits, xanthine-oxidase inhibitors such as allopurinol (Fuchs et al. 1999), or other drugs (Klebovitch et al. 1995). NAT activities are affected by anti-inflammatory drugs. Of note, acetaminophen is an inhibitor of NAT2 in vivo (Rothen et al. 1998).

Discordances between caffeine and dapsone phenotyping data, and between NAT2 phenotyping status and genotyping have been observed in acutely ill patients infected with HIV (O'Neil et al. 2000), which may be due partly to non-detection of rare NAT2 alleles (Alfirevic et al. 2003).

Modifications of the Method

Some recent references for other used NAT2 phenotyping tests can be found for dapsone in Alfirevic et al. (2003), O'Neil et al. (2000), Queiroz et al. (1997), for sulfamethazine in Hadasova et al. (1996) and Meisel et al. (1997), and for procainamide in Okumura et al. (1997) and Mongey et al. (1999).

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Uridine Diphosphate Glucuronosyltransferases

Glucuronidation is a potent detoxification pathway. The uridine diphosphate glucuronosyltransferases (UGTs) are involved in the biotransformation of endogenous substances (bilirubin, biliary acids, and steroid hormones) and numerous drugs and carcinogens. Currently, 20 functional UGTs have been characterized with activity mainly expressed in the liver and the GI tract. There are three subfamilies: UGT1A, UGT2A, and UGT2B, with distinct but broad overlapping substrate specificity existing for the different isoforms of each family. UGT1A1 is the most abundant UGT in the liver. Human diseases related to deficient UGT1A1 alleles are the well-characterized inherited unconjugated hyperbilirubinemias, including the Gilbert's syndrome that affects 6–12% of Caucasian subjects. Exhaustive reviews on roles, tissue patterns of expression, and pharmacogenomics of UGTs can be found in papers from Tukey and Strassburg (2000), Fischer et al. (2001), Guillemette (2003), and Wells et al. (2004).

A decreased clearance has been observed for some drugs metabolized by glucuronidation in patients with Gilbert's syndrome. A clinically significant impact of UGT polymorphism has to date is only demonstrated for some anticancer agents: clearly for irinotecan, and with contradictory results for flavopiridol (Zhai et al. 2003). UGT1A1 and UGT1A9 are involved in the glucuronidation of the active metabolite SN-38 of irinotecan. The presence of the deficient UGT1A1*28 variant (most frequent variant as compared to UGT1A9 variants) has been clinically linked to a decrease in SN-38 glucuronidation rate and to an increased occurrence of serious side effects, mainly severe diarrhea and neutropenia (Ando et al. 1998; Innocenti et al. 2004; Iyer et al. 2002; Paoluzzi et al. 2004). Variants of UGT1A7 were reported to affect SN-38 glucuronidation but only in vitro (Villeneuve et al. 2003). Other factors, such as polymorphism in drug transporter P-glycoprotein and renal excretion, may play a role in the complex disposition pattern of irinotecan.

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Methyltransferases

There are at least four enzymes catalyzing S-, N- and O-methylation using S-adenosylmethionine, but only TPMT polymorphism has been found to have important clinical consequences. To date, no endogenous substrate of TPMT is known. TPMT is involved in the metabolism of mercaptopurine, azathioprine and thioguanine, narrow therapeutic index drugs in use for the treatment of patients with neoplasia or autoimmune disease, or of transplant recipients. About 0.3% of Caucasian subjects have no detectable enzyme activity and 10% intermediate activity (McLeod and Evans 2001). Four alleles TPMT*2, TPMT*3A, TPMT*3B, and TPMT*3C account for 80–95% of Caucasians with intermediate or low enzyme activities. Patients with low inherent TPMT activity are at great risk for severe potentially life-threatening myelosuppressive toxicity with treatment by the above-mentioned drugs, whereas subjects with very high activity might be underdosed (Zhou 2006). Patients with two nonfunctional variant TPMT alleles should receive 5–10% of drug standard doses. TPMT genotyping has proved its usefulness in individualizing mercaptopurine dose in patients, and can replace the phenotyping test: measurement of the erythrocyte enzyme activity, based on the in vitro conversion of 6-mercaptopurine to 6-methylmercaptopurine or 6-thioguanine to 6-methylthioguanine (Innocenti et al. 2000; Evans 2004). A cut-off concentration of 45.5 nmol thioguanine/gHb h^{-1} for this TPMT phenotyping test has been proposed for assessing the need of the genotyping test (Wusk et al. 2004).

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Glutathione S-transferases and Sulfotransferases

Glutathione and sulfatation conjugations are important pathways for generally detoxifying endogenous substrates and xenobiotics (Commandeur et al. 1995). However, some produced metabolites (i.e., mercapturic acids, O-sulfo conjugates) are toxic by different mechanisms, often by reaction with DNA and other cellular nucleophils.

Eight classes of glutathione- S-transferases (GSTs) have been described. The role of the glutathione pathway and the impact of enzyme polymorphism have been highlighted for detoxification and some disease susceptibility, and routine phenotyping of some GSTs exists for clinical safety measurement, but currently there is no evidence of genotyping or phenotyping usefulness for drug dosage adjustment (Hayes and Strange 2000; Tetlow et al. 2004). GSTs are involved in the detoxification of chemotherapeutics, including platinum derivatives. Polymorphisms in the GSTP1 genotype might become a powerful tool to predict oxaliplatin-induced cumulative neuropathy (Lecomte et al. 2006).

Soluble sulfotransferases are involved in the sulfonation of endogenous substrates (notably steroids, neurotransmitters, and eicosanoids) and numerous xenobiotics (i.e., acetaminophen, and organic-platin anticancer agents). The presence of some sulfotransferases variants could be associated with some cancer risk. Phenotyping tests have been developed for some forms (SULT1A and SULT1A3) by measuring platelet sulfotransferase activity (Glatt and Meinl 2004).

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