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MiniReview

Assessment of CYP1A2 Activity in Clinical Practice: Why, How, and When?

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Abstract: The cytochrome P450 enzyme CYP1A2 mediates the rate-limiting step in the metabolism of many drugs including theophylline, clozapine, and tacrine as well as in the bioactivation of procarcinogens. CYP1A2 activity shows both pronounced intra- and interindividual variability, which is, among other factors, related to smoking causing enzyme induction, to drug intake and to dietary factors which may result in induction or inhibition. In contrast to these exogenous factors, genetic influences on enzyme activity seem to be less pronounced. Therefore, phenotyping of CYP1A2, i.e. the determination of the actual activity of the enzyme *in vivo*, represents a useful approach both for scientific and clinical applications. CYP1A2 is almost exclusively expressed in the liver. Since liver tissue cannot be obtained for direct phenotyping, a probe drug which is metabolized by CYP1A2 has to be given. Proposed probe drugs include caffeine, theophylline, and melatonin. Caffeine is most often used because of the predominating role of CYP1A2 in its overall metabolism and the excellent tolerability. Various urinary, plasma, saliva, and breath based CYP1A2 caffeine metrics have been applied. While caffeine clearance is considered as the gold standard, the salivary or plasma ratio of paraxanthine to caffeine in a sample taken approximately 6 hr after a defined dose of caffeine is a more convenient, less expensive but also fully validated CYP1A2 phenotyping metric. CYP1A2 phenotyping is applied frequently in epidemiologic and drug-drug interaction studies, but its clinical use and usefulness remains to be established.

The human cytochrome P450 enzyme CYP1A2 plays an important role in the metabolism of several clinically used drugs. It is one of the major P450 enzymes and accounts for approximately 13% of the total content of this enzyme group in the human liver (Shimada *et al.* 1994). CYP1A2 mRNA content shows an up to 40-fold variability between individuals (Schweikl *et al.* 1993) and corresponding variability of enzyme activity and drug metabolism (Potkin *et al.* 1994).

Genotyping and phenotyping are the two methods that are used today to assess the *in vivo* activity of drug-metabolizing enzymes. Genotyping works well to predict enzyme activity if a major fraction of variability is attributable to known polymorphisms. Although it allows cost- and time-effective characterization of many significant genes at once, it will fail to provide reasonably exact estimates of enzyme activity when other major factors of influence (e.g. liver disease, enzyme induction/inhibition) are prevalent. To a great extent, this applies to CYP1A2. Consequently, the optimal method of describing actual enzyme activity would be phenotyping with a carefully selected probe compound or endogenous substance which provides the most clinically relevant information, because it is a reflection of the combined effects of genetic, environmental and endogenous factors on enzyme activity (Streetman *et al.* 2000).

The following sections of this article therefore discuss reasons, available methods and potential clinical applications for CYP1A2 phenotyping.

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Why might CYP1A2 phenotyping be helpful?

The elimination of a still growing list of drugs such as theophylline (Sarkar *et al.* 1992), tacrine (Spaldin *et al.* 1994), clozapine (Bertilsson *et al.* 1994), olanzapine (Callaghan *et al.* 1999) etc. depends mainly on the activity of CYP1A2 as these drugs are predominantly metabolized by this enzyme. Many more drugs are known where CYP1A2 is involved at least to a potentially clinically relevant extent (table 1a) or seems to play a minor role (table 1b). Moreover, the enzyme is important for the metabolism of endogenous substrates (Guengerich 1993) and for the elimination of environmental toxins as well as for the activation of many environmental carcinogens including dietary heterocyclic amines, certain mycotoxins, tobacco-specific nitrosamines, and aryl amines (Eaton *et al.* 1995).

The observed variability in activity of CYP1A2 is based on constitutional and genetic factors and enzyme induction/inhibition. Depending on the population studied and the method used, interindividual variability may be up to 60-

Table 1.

1a. Drugs which are metabolized by CYP1A2 to an extent suggesting clinical relevance.

Drug	Fraction of elimination attributable to CYP1A2*, metabolite formed via CYP1A2	Other CYPs involved in the metabolism of the drug	Evidence of involvement of CYP1A2 in the metabolism comes from studies	Ref. (examples)
Caffeine	90%, paraxanthine, others	CYP2E1, (CYP3A4/3A5)	in patients (undergoing liver transplantation) in volunteers *in vitro*	Fuhr *et al.* (1996) Kalow & Tang (1993) Gu *et al.* (1992)
Clozapine	22–30%, norclozapine	CYP2C19, CYP3A4, (CYP2C9, CYP2D6)	in patients with psychosis in volunteers *in vitro*	Özdemir *et al.* (2001a & b) Bertilsson *et al.* (1994) Olesen & Linnet (2001)
Flutamide	major pathway, 2-hydroxyflutamide	CYP3A4	caffeine test in patients on flutamide *in vitro*	Ozono *et al.* (2002) Shet *et al.* (1997)
Frovatriptan	up to 27–49%, desmethylfrovatriptan	none	no original data published reviewed in 2002	Anonymous (2003) Buchan *et al.* (2002)
Lidocaine	major route (~80%), 2 active metabolites	CYP3A4	in patients with liver impairment in volunteers *in vitro*	Orlando *et al.* (2004) Orlando *et al.* (2004) Wang *et al.* (1999)
Melatonin (also endogenous substrate)	90%, 6-OH melatonin, (*N*-acetylserotonin)	CYP2C19, CYP1A1, CYP1B1	in a patient on fluvoxamine in volunteers *in vitro*	Grözinger *et al.* (2000) Härtter *et al.* (2001a) Härtter *et al.* (2001b) Ma *et al.* (2004)
Mexiletine (also inhibits 1A2)	7–30%, p- and 2-hydroxymexiletine	CYP2D6	interaction with theophylline in case reports *in vitro*	Kendall *et al.* (1992) Nakajima *et al.* (1998)
Olanzapine	~50%, 4′-*N*-desmethylolanzapine	CYP2D6	in psychiatric patients in volunteers *in vitro*	Hiemke *et al.* (2002) Carrillo *et al.* (2003) Shirley *et al.* (2003) Ring *et al.* (1996)
Ropivacaine	~39%, 3-OH-ropivacaine	CYP3A4	in volunteers *in vitro*	Arlander *et al.* (1998) Ekström & Gunnarsson (1996)
Tacrine	very important (>90%?), 1-, 2-, and 4-OH-tacrine, several other metabolites	none identified	in patients with dementia in volunteers *in vitro*	Fontana *et al.* (1998) Larsen *et al.* (1999) Spaldin *et al.* (1994 & 1995)
Theophylline	75%, 3-methylxanthine, 1-methylxanthine, others	CYP2E1, CYP1A1, (CYP3A4/3A5)	in patients (treated with theophylline) in volunteers *in vitro*	Obase *et al.* (2003) Rasmussen & Brøsen (1997) Gu *et al.* (1992); Ha *et al.* (1995)
Tizanidine	major pathway, unknown	none	in volunteers *in vitro*	Granfors *et al.* (2004 b & c) Granfors *et al.* (2004 a)
Triamterene	up to 100%, p-hydroxytriamterene	(CYP3A, CYP2A6, CYP2D6)	*in vitro*	Fuhr *et al.* (2005)
Zolmitriptan	major pathway, N-desmethylzolmitriptan & zolmitriptan-N-oxide	none	*in vitro*	Wild *et al.* (1999)

* this fraction is derived from *in vitro* studies, it may vary widely, and will, in most cases be increased when CYP1A2 activity is induced. Extrapolations to *in vivo* situations are not recommended, because other factors like non-metabolic clearance should be taken into account.

fold (Sesardic *et al.* 1988), but is commonly between 5- and 15-fold (Schrenk *et al.* 1998; Tantcheva-Poór *et al.* 1999). Although there is evidence that genetic factors have major influence on observed enzyme activity (Rasmussen *et al.* 2002), the role of polymorphisms identified to date is still being discussed (Sachse *et al.* 1999; Aklillu *et al.* 2003; Obase *et al.* 2003; Van Der Weide *et al.* 2003; Sogawa *et al.* 2004). While polymorphisms in coding regions are rare and only occasionally seem to decrease CYP1A2 activity *in vivo* (e.g. a point mutation in the donor splice site of intron 6, named CYP1A2*7 (Allorge *et al.* 2003)), some mutations in the putative promoter region are accused to cause changes in inducibility of CYP1A2. Japanese smokers with a point mutation in the promoter region classified as *1C

Table 1.

1b. Drugs metabolized (at least to some extent) by CYP1A2 where the clinical relevance is minor, unclear or unknown.

Drug	Fraction of elimination attributable to CYP1A2*, metabolite formed via CYP1A2	Other CYPs involved in the metabolism of the drug	Evidence of involvement of CYP1A2 in the metabolism comes from studies	Ref. (examples)
Acetaminophen	negligible, *N*-acetyl-p-benzoquinone imine	CYP2E1, CYP3A4	evidence reviewed in 2000	Manyike *et al.* (2000)
Almotriptan	negligible, M4-metabolite	CYP3A, CYP2C8, CYP2C19, CYP2D6	*in vitro*	Salva *et al.* (2003)
Amitriptyline	minor, nortriptyline	CYP2C19, CYP2D6, CYP3A4, CYP2C9	*in vitro*	Venkatakrishnan *et al.* (1998)
Clomipramine	not quantified, *N*-desmethylclomipramine	CYP3A4, CYP2D6	case report on an interaction *in vitro*	Fisman *et al.* (1996) Nielsen *et al.* (1996)
Cyclobenzaprine	relatively important, desmethylcyclobenzaprine	CYP3A4, (CYP2D6)	*in vitro*	Wang *et al.* (1996)
Fluvoxamine (also inhibits 1A2)	unclear, fluvoxamino acid	CYP2D6	in volunteers	Carrillo *et al.* (1996)
Imipramine	~30%, higher in 2C19 poor metabolizers, desipramine	CYP3A4 (?), CYP2C19, CYP2D6, CYP2C18	*in vitro* *in vitro*	Lemoine *et al.* (1993) Koyama *et al.* (1997)
Maprotiline	17%, desmethylmaprotiline	CYP2D6	*in vitro*	Brachtendorf *et al.* (2002)
Naproxen	relatively important, O-desmethylnaproxen	CYP2C9, CYP2C8?	*in vitro* *in vitro*	Tracy *et al.* (1997) Miners *et al.* (1996)
Oestrogen	less than 21%, 2 hydroxylated metabolites	CYP3A, CYP2C9	*in vitro*	Kerlan *et al.* (1992)
Ondansetron	minor, 7- and 8-OH-ondansetron	CYP1A1, CYP2D6, CYP3A	*in vitro*	Dixon *et al.* (1995)
Perphenazine	20%, *N*-dealkylated metabolite	CYP3A4, CYP2C19, CYP2D6	*in vitro*	Olesen & Linnet (2000)
Propafenone	minor pathway, *N*-depropylpropafenone	CYP3A4, CYP2D6.	in patients, *in vitro*	Botsch *et al.* (1993)
Propranolol	probably important, 4-hydroxypropranolol	CYP2D6, CYP2C19 (?)	*in vitro* *in vitro*	Johnson *et al.* (2000) Yoshimoto *et al.* (1995)
Riluzole	unknown, N-hydroxyriluzole	CYP1A1, others?	*in vitro*	Sanderink *et al.* (1997)
Thioridazine	unclear, at least 3 metabolites	CYP2C19, CYP2D6	interaction with fluvoxamine in patients	Carrillo *et al.* (1999)
Verapamil	minor pathway, norverapamil, D617	CYP3A4, CYP2C-family	in volunteers *in vitro*	Fuhr *et al.* (2002) Kroemer *et al.* (1993)
(R)-Warfarin	minor pathway, 6-OH- & 8-OH-(R)-warfarin	CYP3A4, CYP2C9, (CYP2C19)	case report on interaction with fluvoxamine interaction potential reviewed *in vitro*	Limke *et al.* (2002) Lehmann (2000) Kaminsky & Zhang (1997)
Zileuton (also inhibits 1A2)	minor, hydroxylated metabolite	CYP3A4, CYP2C9	*in vitro*	Machinist *et al.* (1995)
Zotepine	minor, 2-hydroxyzotepine	CYP3A4, CYP2D6	*in vitro*	Shiraga *et al.* (1999)

* this fraction is derived from *in vitro* studies, it may vary widely, and will, in most cases be increased when CYP1A2 activity is induced. Extrapolations to *in vivo* situations are not recommended, because other factors like non-metabolic clearance should be taken into account.

allele showed a decreased CYP1A2 activity (or less induction?) compared to smokers without this mutation (Nakajima *et al.* 1999). In smokers homozygous for the *1F allele, a 1.6 times higher induction was observed (Sachse *et al.* 1999). However, the presence of *1F alleles did not influence CYP1A2 activity in non-smokers and patients with colorectal cancer (Sachse *et al.* 2003). In Ethiopians and Saudi-Arabians, a point mutation in the promoter region was identified and functionally characterized which lead to both a lower enzyme activity and inducibility (Aklillu *et al.* 2003).

In this context, it is of interest to note that these mutations are relatively frequent in the ethnicity where they have been first described, but only occasionally found in groups of other ethnic background.

On the other hand, induction and inhibition has been established as a main feature of P450s and many factors contributing to variability via this mechanism have been identified. Intake of polycyclic aromatic hydrocarbons (e.g. via cigarette smoking), caffeine, cruciferous vegetables, heavy exercise, grilled meat, and certain drugs such as omeprazole and carbamazepine induce CYP1A2. In the case of TCDD intoxication, an at least 10-fold induction has been reported (Vistisen *et al.* 1991; Nousbaum *et al.* 1994; Kall & Clausen 1995; Le Marchand *et al.* 1997; Parker *et al.* 1998; Abraham *et al.* 2002). Many other drugs such as fluvoxamine, quinolone antibiotics and oral contraceptives act as inhibitors (Gardner *et al.* 1983; Fuhr *et al.* 1992; Christensen *et al.* 2002). Factors of influence on CYP1A2 activity in a healthy population have also been systematically investigated in some studies (table 2). It has to be emphasized that, besides smoking, concomitant intake of drugs which are inhibitors, inducers or even merely competing substrates for CYP1A2 represents one of the most prominent and frequent factors of influence on CYP1A2 activity. However, it is beyond the scope of this article to review the knowledge of the influence of comedication on CYP1A2 activity.

Increased enzyme activity (disregarding the underlying cause) is a mixed blessing. It is expected to shorten exposure to xenobiotics (e.g. environmental toxins) by accelerated catalysis of metabolic steps in elimination. In case of involvement in the metabolism of drugs, induction can cause nonresponse to therapy due to elimination faster than expected of the substances and consequently lowered *in vivo* drug concentrations, as it is the case for theophylline which is more rapidly metabolized in smokers (Gardner *et al.* 1983). In contrast, for inactive substances that are biotransformed to active metabolites, accelerated enzyme activity may result in higher concentrations of the active (possibly carcinogenic) component (Fuhr 2000). According to this consideration, higher CYP1A2 activity has in some studies been associated with a higher prevalence of some forms of cancer (Lang *et al.* 1994; Horn *et al.* 1995).

Similar implications apply to enzyme inhibition: decreased enzyme activity slows down elimination of xenobiotics and can result in increased *in vivo* drug concentrations and exaggeration of drug effects (Pelkonen *et al.* 1998).

Due to the variability in activity, and the enzyme's involvement in the metabolism of a broad range of xenobiotics including environmental compounds, (pro)carcinogens and drugs (Shimada *et al.* 1994; Eaton *et al.* 1995), knowledge of CYP1A2 activity and factors of influence is of both scientific and clinical interest.

Phenotyping can help scientifically in finding the aetiology of diseases (e.g. cancer) or to identify and quantify sources of enzyme activity variation in large populations. To aid the clinician, such data may in turn be used to predict biotransformation capacity and to individualize drug therapy (Tantcheva-Poór *et al.* 1999).

For the selection of drugs and initial doses in patients, taking known factors of influence on enzyme activity into account might suffice to yield a good estimate of activity for some enzymes. For example, a concordance rate of over 98% for genotype and phenotype of thiopurine S-methyltransferase has been found (Schaeffeler *et al.* 2004). But in the case of CYP1A2, a great deal of variability remains unexplained. Even if every single factor was identified and quantified, cumulative effects of genetics, lifestyle, inhibition and induction cannot simply be totalled, but are rather difficult to predict. Phenotyping represents here a suitable method to shed light on the actual enzyme activity.

In this manner, phenotyping might be in the direct interest of an individual patient. Assessing his metabolic capacity before or during drug therapy can help to find the optimal individual dose or give hints at the cause of adverse effects or nonresponse. Moreover, phenotyping of an enzyme mainly expressed in the liver might also provide an opportunity for a safe follow-up liver function test in patients with liver disease (Lelouet *et al.* 2001).

Table 2.

Factors determining CYP1A2 activity in healthy populations*. Representative references were selected for quantitative data. Some values were calculated from data given in literature (Sinha *et al.* 1994; Kall *et al.* 1996; Kashuba *et al.* 1998; Fontana *et al.* 1999; Tantcheva-Poór *et al.* 1999; Zaigler *et al.* 2000b).

Source of variation	Mean resulting change of CYP1A2 activity determined by phenotyping
Cigarette smoking	dose dependent, 1.22-fold, 1.47-fold, 1.66-fold and 1.72-fold for 1–5, 6–10, 11–20 and >20 cigarettes smoked per day
Coffee	1.45-fold per litre of coffee drunk daily
Body mass index	0.99-fold per $kg \cdot m^{-2}$
Female gender	0.90-fold
Oral contraceptives	0.72-fold
Menstrual cycle	1.03-fold up to 1.10-fold (mid-luteal relative to mid-follicular phase)
500 g broccoli daily	1.19-fold
Meat pan-fried at high temperatures	1.40-fold (highly variable, after a controlled 7 day diet)
Chargrilled meat	1.89-fold (variable, after a controlled 6 day diet)

* In addition, many drugs have a major effect (inhibition, induction) on CYP1A2 activity; however, it is beyond the scope of this article to list all these drugs.

How is CYP1A2 phenotyping done?

Determination of enzyme activity can easily be done by performing *ex vivo* function tests with tissues that express the relevant enzyme. However, CYP1A2 is not contained in blood cells or otherwise fairly accessible body compounds of the patient, but is mainly confined to the liver. Therefore, liver biopsy is the only, but for ethical and practical reasons a hardly justifiable way of performing such tests (Kalow & Tang 1993). Instead, different probe drugs or endogenous substances (phenacetin, caffeine, theophylline, melatonin)

have been introduced to estimate enzyme activity on the basis of concentrations of the substances and their metabolites in diverse matrices such as plasma, saliva, breath, or urine after administration of the probe. Apparently, CYP1A2 only possesses one substrate binding site which is relatively rigid (Anzenbacher & Hudecek 2001), so there is no need to use more than one substrate for phenotyping. Since phenacetin is no longer used as a drug because of its toxicity, it will not be discussed here.

Caffeine is the most commonly used substance for CYP1A2 phenotyping. The first step in its metabolism is almost exclusively mediated by CYP1A2, it is available from diverse sources (coffee, soft drinks, tablets), the use is without relevant risk in habitual coffee drinkers, and concentrations in biological matrices can be determined using a simple HPLC method (Fuhr & Rost 1994). The disadvantage is that dietary intake of methylxanthines (coffee, black tea, cola, chocolate, etc.) should be avoided up to 36 hr before and during the phenotyping procedure. Furthermore, metabolism of caffeine is rather complex, involving several different P450 enzymes in minor pathways, and some metabolites of caffeine are both substrate and product of CYP1A2, which makes timing of the samples critical. Metabolism of theophylline is less complex, but 1-demethylation, the only metabolic step exclusively attributable to CYP1A2 (Streetman *et al.* 2000), just represents a minor metabolic pathway *in vitro* (Gu *et al.* 1992). A comparative study (Rasmussen & Brøsen 1997) showed no benefit in the use of theophylline over the use of caffeine as a phenotyping probe. Orally given melatonin may also serve as a probe drug for the assessment of CYP1A2 activity. It is rather nontoxic, but its suitability and robustness as a phenotyping probe has yet to be assessed in a larger number of subjects (Härtter *et al.* 2001a).

Due to the nature of the approach, phenotyping will only provide an estimate of the actual enzyme activity (Tucker *et al.* 1998). A proposed phenotyping metric should be validated to assess whether it really reflects the actual enzyme activity and is not relevantly influenced by other factors. Watkins (1994) proposed several criteria for valid phenotyping with respect to liver enzymes. These criteria have been extended and applied to probes for CYP3A4 and CYP1A2 (Zaigler *et al.* 2000a). Generally, any phenotyping metric should correlate with the target enzyme activity *in vitro*, in healthy volunteers and in patients, should reflect induction and inhibition of the target enzyme as well as (severe) liver disease, should be reproducible and not be influenced by other factors. Additionally, low invasiveness, low costs, ease of the procedure and accuracy, ease, and reproducibility of the analytical procedure should be taken into account.

In comparison to theophylline and melatonin, caffeine-based metrics for CYP1A2 activity have more extensively been validated. Caffeine fulfills most, but not all of the validation criteria. It has been shown that CYP1A2 mediated caffeine clearance accounts for more than 95% of overall caffeine elimination from the plasma (Lelo *et al.* 1986; Gu *et al.* 1992; Kalow & Tang 1993), and that praraxanthine formation is virtually exclusively mediated by CYP1A2, but caffeine elimination is also dependent on renal function, moreover, caffeine itself mildly increases urine production and thereby enhances its own elimination. However, it became clear in simulation studies that the effect of caffeine on renal function does not obscure the effect of CYP1A2 on caffeine clearance (Rostami-Hodjegan *et al.* 1996).

Three important issues in assessing the enzyme activity are the way how phenotyping is carried out, the choice of the matrix in which caffeine and its metabolites are quantified, and which pharmacokinetic parameter is used.

It is quite evident that any methylxanthine source should be avoided between the intake of the caffeine test dose and the end of the sampling period. Additionally, most authors agree that before phenotyping, a methylxanthine abstinent period of 12 to 36 hr has to be respected. Although it has not been formally investigated whether this abstinence is really necessary, it seems to be prudent to exclude participants who show high caffeine concentrations before the administration of the caffeine test dose (Tantcheva-Poór *et al.* 1999; Zaigler *et al.* 2000a).

Correlation analyses between immunoreactive CYP1A2 liver content, intrinsic clearance for caffeine-3-demethylation to paraxanthine, and various plasma, saliva, and urine based CYP1A2 metrics have shown the closest correlations between intrinsic clearance and saliva based caffeine clearance estimation ($r=0.82$, $P<0.001$), followed by the plasma paraxanthine to caffeine ratio determined 6 hr after caffeine intake ($r=0.78$, $P<0.001$) and the saliva paraxanthine to caffeine ratio 6 hr after caffeine intake ($r=0.74$, $P<0.001$) in patients undergoing hepatectomy (Fuhr *et al.* 1996). The correlations to immunoreactive CYP1A2 content in the liver corroborated these findings. A comparison between six methods for caffeine based CYP1A2 phenotyping showed that, besides caffeine systemic clearance as the "gold standard" in direct comparison to CYP1A2 activities in liver biopsies (Fuhr *et al.* 1996), the paraxanthine to caffeine ratio determined in plasma or in saliva as a metric for the CYP1A2 mediated partial caffeine clearance to paraxanthine most completely fulfilled validation criteria (Zaigler *et al.* 2000a).

Because of its low invasiveness, the determination of the metabolic ratio in a spot saliva sample seems advantageous (Rostami-Hodjegan *et al.* 1996) and yields meaningful results (Tantcheva-Poór *et al.* 1999). At least six urinary ratios have been used for the assessment of CYP1A2 activity. However, most, if not all of them are prone to be influenced by confounding factors such as renal blood flow, urinary flow, and the activities of other enzymes involved in caffeine biotransformation such as *N*-acetyltransferase 2, xanthine oxidase, and CYP2E1 (Rostami-Hodjegan *et al.* 1996). The caffeine breath-test procedure, one of the oldest tests for the evaluation of cytochrome P450 activities (Wietholtz *et al.* 1981), is limited in its use and usefulness by several factors: ^{13}C-labelled caffeine has to be used which is not readily available, timing of the breath sample is a critical factor

such as physical activity during the test period, and specialized equipment is needed for the measurement of the exhaled $^{13}CO_2$ (Kalow & Tang 1993). Therefore, this method is expensive and only rarely used.

In conclusion, estimation of CYP1A2 activity using a saliva sample approximately 6 hr after a 100–200 mg caffeine test dose and quantifying the molar ratio of paraxanthine to caffeine represents to date the best compromise between validity, reliability, accuracy, costs, and ease of handling (Fuhr & Rost 1994; Rostami-Hodjegan *et al.* 1996; Fuhr *et al.* 1996). However, adherence to a methylxanthine-free diet during and before the test is a critical factor for the reliability of the results. To control for compliance, the analysis of a predose saliva sample and the exclusion of subjects showing values suggesting noncompliance has been proposed (Tantcheva-Poór *et al.* 1999).

When should CYP1A2 phenotyping be used?

Inhibition and induction of P450s are among the most frequent reasons for drug-drug interaction (Lin & Lu 1998). From the industrial and drug safety perspective, the indication for CYP1A2 phenotyping is therefore clear: If there is no definite evidence that a new chemical entity does not affect CYP1A2, an interaction study using a model substrate for this enzyme should be conducted in phase I of the clinical drug development. This is applied, for reasons of practicability and cost-effectiveness often in the form of a cocktail approach, where several enzymes are phenotyped simultaneously with different substrates.

Phenotyping should also be (and is already) used scientifically in the investigation of other factors modulating enzyme activity. However, to achieve comparability and validity in the degree of effects on enzyme activity, it would be helpful if already established and validated phenotyping procedures, such as the paraxanthine to caffeine ratio in blood or saliva were used.

In clinical practice, variability of drug exposure due to variability of enzymes involved in the metabolism would demand that drug dosages are selected for and adjusted to each individual patient. However, individualized drug therapy and especially phenotyping is not (yet) a routine clinical or laboratory procedure. In most cases, standard doses are selected empirically and dose adjustment is done based on clinical observation *a posteriori.*

The success of drug therapy therefore relies mainly on two factors, which are (i) a wide therapeutic index of the given drug, and (ii) near "average" drug metabolism in the patient. If one or both of these are not applicable, therapy is actually in many cases individualized by gradually increasing the dose from a presumably safe level while monitoring either clinical effects or drug concentrations in biological matrices (therapeutic drug monitoring), or preferably both.

For the individualized selection of therapy, it might be advantageous to assess enzyme activity in the patient with the help of phenotyping prior to the start of a therapy. For clozapine and olanzapine, two atypical neuroleptics used to treat schizophrenic patients, the potential value of CYP1A2 phenotyping has been investigated. Both substances are metabolized predominantly by CYP1A2 and abnormally low or high enzyme activity seems to be associated with adverse drugs effects or non-response, respectively. A close relationship between various caffeine metabolic ratios and steady-state concentrations of clozapine and olanzapine has been found. Several authors therefore agree on phenotyping to be potentially useful in individualizing the initial dosage. Moreover, an additional caffeine test gives the benefit of being able to distinguish between noncompliance and non-response, which can hardly be accomplished by determination of plasma drug concentrations alone (Özdemir *et al.* 2001a & b; Carrillo *et al.* 2003; Shirley *et al.* 2003).

Drug dose might have to be adjusted to the patient not only before the start of a therapy, but also after changes in diet, lifestyle, concomitant drug intake or environment. These influences may lead to an extent of change in individual CYP1A2 activity which is in the order of magnitude of interindividual variability. However, changes in CYP1A2 activity are transient and, in contrast to genetic factors, can be modified virtually at random. Cessation of smoking, introduction of strong enzyme inhibitors to the therapy regimen (e.g. fluvoxamine) or even a change in habitual coffee consumption has been shown to modulate CYP1A2 activity to an extent that can lead to adverse drug effects through elevated plasma concentrations (Carrillo *et al.* 1995; Olesen *et al.* 1996; Zullino *et al.* 2002; Faber & Fuhr 2004).

It does not seem feasible to phenotype the patient each time a change in lifestyle occurs that is known to influence CYP1A2 activity, as it is not expected to give any benefit over the combination of clinical monitoring, experienced dose adjustment and therapeutic drug monitoring. However, CYP1A2 phenotyping may be considered when therapeutic drug monitoring is not established for a given drug, or adverse drug effects or non-response cannot be clearly attributed to other causes. In this manner, it can be of use for the individual patient as well as for the discovery of relevant drug interactions or other influencing factors. There is one theoretical caveat in the interpretation of phenotyping results when drugs are taken concomitantly which are substrates for the same enzyme: depending on the different affinity of substrates to CYP1A2 and on differences in occupation of the enzyme's active site, one drug may act as a competitive inhibitor of the metabolism of another. Whether such a competition really may lead to erroneously low caffeine metabolic ratios during phenotyping has to date not been scrutinized.

Consequently, CYP1A2 phenotyping may find its place in clinical practice in addition to clinical monitoring, therapeutic drug monitoring, and the consideration of patient characteristics as a tool for individualizing drug therapy, compliance control and as a help in finding the cause of adverse drug reactions or nonresponse (table 3).

It has to be admitted that no studies have been conducted to date regarding the use of this instrument in clinical situ-

Table 3.

Potential applications for CYP1A2 phenotyping.

CYP1A2 phenotyping may be useful in
• volunteers before and during drug-interaction and drug metabolism studies
• volunteers for phase I studies
• healthy and / or diseased participants in epidemiologic studies
• patients before receiving drugs which are mainly metabolized by CYP1A2 for *a priori* dose individualization
• patients having adverse reactions possibly caused by CYP1A2 inhibition
• patients not responding to drugs mainly metabolized by CYP1A2
• patients with liver diseases for an additional evaluation and/or follow up of liver function
• dose adaptations when patients stop smoking and receive drugs metabolized by CYP1A2

ations. Consequently, the benefit is yet unclear. Moreover, the balance between therapeutic and adverse effects has to be found by clinical monitoring, as different patients need different *in vivo* drug concentrations.

Conclusion

Because CYP1A2 activity is highly variable both within and between individuals, because known polymorphisms do not seem to explain the variability sufficiently, and because induction e.g. by tobacco consumption is frequent, phenotyping is the most appropriate approach to estimate an individual's CYP1A2 activity. Among the various metrics proposed for CYP1A2 phenotyping, a saliva or plasma based determination of the paraxanthine to caffeine ratio roughly 6 hr after intake of a defined amount of caffeine is to date the most convenient of the fully validated methods. For scientific purposes, this phenotyping approach has proven useful in epidemiological and in drug-drug interaction studies. However, it is rarely used in clinical routine, although for several clinically important drugs the therapeutic efficacy and undesired effects seem to be related to CYP1A2 activity. CYP1A2 phenotyping may be helpful in the choice of initial doses or in the differentiation between noncompliance and nonresponse in patients treated e.g. with theophylline, clozapine, or olanzapine, but studies on the benefit of routine CYP1A2 phenotyping in such patients are lacking. In view of the large number of drugs metabolized at least in part by CYP1A2 and of the large variability in CYP1A2 activity, studies further investigating the effect of mutations on the enzyme activity and inducibility in target populations are needed as well as studies in patients linking caffeine-based phenotype, genotype, and the clinical effects of drugs.

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